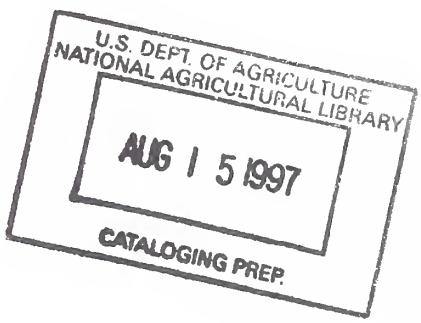
Heliothis/Helicoverpa

1993 Supplement to the Five-Year National Research Action Plan for Development of Suppression Technologies

First Annual Review
Held in Junction, Texas
November 8–11,1993



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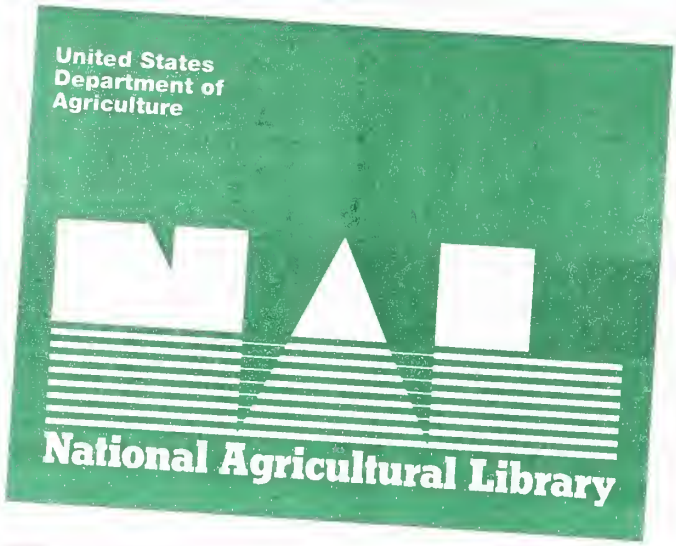

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Issued October 1994

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Acknowledgments

The National Program Leaders, and Steering Committee sincerely appreciate the contributions of all the participants. We especially appreciate the efforts of Jesus Esquivel, Ritchie Eyster, Henery Marshall, Denise Mayfield, Mike O'Neil, Irene Sanders, Paul Schlieder, and Dale Spurgeon for their help in local arrangements, accommodations, refreshments, etc.

FOREWORD

This ARS National *Heliothis/Helicoverpa* Working Conference progress report details the first annual review of the revised 1991 (published February 1992) 5-year research action plan and contains a compilation of progress reports, research summaries, work plans, publications and presentations by ARS scientists. The primary goal of the National *Heliothis/Helicoverpa* research program for development of suppression technologies is to provide the necessary research through a cooperative team effort that will lead to the development of environmentally-sound, economical, and publicly-acceptable technologies for management of this pest complex.

The first *Heliothis/Helicoverpa* strategic planning conference was held in October 1985 in Memphis, Tennessee. It was devoted to the identification of critical research needs and a review of the ARS research effort by location, as well as an assessment of the number of scientists involved in research on this pest complex. A second ARS-wide working conference with the goal to develop a revised research action plan was held on September 16-19, 1991, in San Antonio, Texas. The published report of the conference detailed an updated 5-year research action plan for development of suppression and management technologies for these pests. The research action plan was categorized into six major research action areas and identified/confirmed high priority research needs : (a) host-plant resistance; (b) chemical control and pesticide application technology; (c) ecology and population dynamics; (d) behavior modifying chemicals; (e) biological control; and (f) genetics, molecular biology, and basic physiology.

A third ARS-Wide Working Conference was held on November 8-11, 1993, in Junction, Texas to review research progress of the 5-year research action plan since 1991. At this conference, co-coordinators for each of the six action areas provided progress reports on the research action plan's lead arrays, as well as research summaries which are included in this report. The National Research Action Plan provides a dynamic mechanism to ensure program focus and a framework for a unified effort.

The NPS expresses its gratitude to all working conference attendees for participating in the proceedings of the conference and in preparing the comprehensive progress reports and research summaries. We are especially indebted to the conference organizers and to the representatives from CSRS, universities, commodity groups, and industries for their interactions and invaluable contributions.

James R. Coppedge
National Program Staff
Applied Entomology

Robert M. Faust
National Program Staff
Fundamental & Molecular Entomology

EXECUTIVE SUMMARY

The *Heliothis/Helicoverpa* pest complex has a world-wide distribution and contains some of the most serious pests to agriculture. *Heliothis virescens* and *Helicoverpa zea* are pests on a wide variety of crops including cotton, corn, soybean, lettuce, tomato, tobacco, ornamental and other economic plants in the U.S. These pests are responsible for a loss of about $2 billion annually in reduced yield and control costs. Current methods of control rely on the field-to-field applications of synthetic chemical insecticides. Development of integrated control strategies that reduce dependency on chemical insecticides is the primary focus for the ARS National research program on *Heliothis/Helicoverpa*.

A *Heliothis/Helicoverpa* workshop was held on September 16-19, 1991 in San Antonio, TX, and a 5-year research action plan for development of management and suppression technologies for these pests was published. This research action plan was categorized into six major research action areas: (a) host-plant resistance; (b) chemical control and pesticide application technology; (c) ecology and population dynamics; (d) behavior-modifying chemicals; (e) biological control; and (f) genetics, molecular biology, and basic physiology. The research program includes extensive collaboration with federal and state agencies, universities, and industry.

A *Heliothis/Helicoverpa* action plan progress review was held on November 8-11, 1993, in Junction, TX. Individual progress reports as well as action area summaries and additional research recommendations are included in this supplement to the action plan.

Some significant accomplishments highlighted during the progress review included: (1) identification of several commercial hybrid corn lines with high levels of antibiosis against corn earworm larvae;(2) identification of a new chemical, "popsin" that suppresses corn earworm growth and development; (3) improved formulation technology for numerous commercially available chemical and biorational insecticides; (4) development of DNA markers to distinguish geographical populations of corn earworm; (5) improvement in pheromone formulation technology for simultaneous mating disruption of corn earworm and tobacco budworm; (6) characterization of association between volatiles from infested plants and performance and behavior of adult parasitoids; (7) demonstration of the nematode, *Steinernema riobravis* as a biological control agent for prepupae and pupae of corn earworm; (8) characterization of a rickettsia-like organism as a factor in backcross sterility of tobacco budworm; and (9) a chemical taxonomic method to distinguish larvae of corn earworm from those of tobacco budworm.

A list of high priority research needs was generated by the research action area teams. These included: (1) development of a marketing strategy for host-plant resistance traits that are currently "on the shelf"; (2) research on the use of application technology in the early phases of the research programs; (3) increased efforts on the identification and development of biologically-based control agents for area-wide management; (4) improvement in methods to assess pesticide spray deposition and drift in terms of environmental/ecological impacts; (5) development of rapid and inexpensive immunological techniques to distinguish immature stages of corn earworm and tobacco budworm for decision-based chemical application; (6) migration and dispersal of resistant populations; (7) improved understanding of *Heliothis/Helicoverpa* ecology and population dynamics; (8) identification of suitable markers for identification of the origin of immigrant moths; (9) determination of the effects of host plants on adult reproduction and migration; (10) population models compatible with area-wide management strategies; (11) development of new sampling technologies suitable for the estimation of adult female and larval population densities; (12) determination of efficacy of sex pheromones for mating disruption; (13) optimization of pheromone blends and dispensers for trapping systems; (14) methods for year-round conservation of natural enemies and early season augmentation of natural enemies; (15) development of mass rearing and quality control of both host insects and natural enemies; and (16) development of automated sexing systems to separate males from females during early developmental stages.

ANNUAL REVIEW OBJECTIVES

Periodic research progress reviews are an integral part of the 5-year National Research Action Plan for Development of Suppression Technologies for *Heliothis/Helicoverpa*. The overall objective of this program review was to examine the progress as well as the current and proposed research activities in relation to goals, objectives and priorities of the ARS 5-year National Research Action Plan. Specific objectives were as follows.

1. Make an assessment of how ARS research activities are meeting objectives and action agency needs.

2. Identify areas where research is still lacking.

3. Provide answers to the questions:

   a. Are all the needs and priorities outlined in the 1991 Action Plan still relevant.
   b. Are there new priorities and needs that have emerged and, if so, what are they.
   c. If there are new priorities, how can they best be addressed, i.e., what shifts and/or adjustments within the ARS program could or should be considered.

4. Provide recommendations, where appropriate, on specific short- and long-term goals.

Progress Reports

Action Area I. Host Plant Resistance

Coordinators: Lavone Lambert and Billy Wiseman

INVESTIGATOR'S NAME(S):		J. A. Eash, C. E. Elliger and A. C. Waiss, Jr.
AFFILIATION & LOCATION:		USDA, ARS, WRRC, Albany, CA 94710
ACTION AREA:	1.	Host Plant Resistance
LEAD ARRAY:	1.1	Develop crop cultivars and/or germplasms with high resistance to reduce numbers of and damage from *Heliothis/Helicoverpa* spp.
SAFEGD ARRAY:	1.1.1	Identify and/or develop new sources of resistance that impact on populations of *Heliothis/Helicoverpa* spp.
OPTIM ARRAY:	1.1.2a	Determine the effectiveness of the resistant cultivar and/or germplasm in reducing populations of *Heliothis/Helicoverpa* spp.
	1.1.2b	Determine the interactions of plant resistance to *Heliothis/Helicoverpa* spp. with other methods of integrated pest management.
SUPPL ARRAY:	1.1.3	Develop plant resistance technology that would impact or alter the course of action of any of the other arrays.

DATES COVERED BY REPORT: September 1991-June 1993.

PROGRESS REPORT: Ten species from the *Solanaceae* family of plants were tested for *Helicoverpa zea* resistance. After several repeated experiments, significant resistance to *H. zea* and *Leptinotarsa decemlineata* (Colorado potato beetle) were identified in several species of *Petunia* and *Physalis*. Since these plants are related to potato and tomato within the *Solanaceae*, plans were made to transfer the insect resistant genes to the commercially important crops. Attempts were made to introduce *H. zea* resistant genes from *Petunia* and *Physalis* to tomato and potato by protoplast fusion. Some of the putative transgenic plants are being evaluated for insect resistance. Over sixty petuniasterones and petuniolides were isolated from several *H. zea* resistant species of *Petunia* and their chemical structures determined. Similarly, ten steriods glycosides have been isolated and identified from *Physalis peruviana.* At levels as low as 2.5 ppm, these compounds have been shown to reduce the larval growth of *H. zea* to one-half that of larvae grown on control diet. While the antigrowth activity of these compounds is well documented, the exact nature of the resistance mechanism is unknown.

FY94 & FY95 WORK PLANS: Since the chemical bases of *Helicoverpa* resistance in *Petunia* and *Physalis* have been established, part of the chemical expertise will be diverted to chemical analysis of the putative transgenic hybrids that are insect resistant. The remaining chemical effort will be redirected to establish the chemical nature of *H. zea* resistance in soybean. This work will be performed in cooperation with Dr. Lavone Lambert, ARS, Stoneville, Mississippi. The introduction of insect resistance genes from *Petunia* and *Physalis* to tomato and potato will continue.

INVESTIGATOR'S NAME(S):		D. M. Jackson
AFFILIATION & LOCATION:		USDA, ARS, CRL, Oxford, NC
ACTION AREA:		Host Plant Resistance
LEAD ARRAY:	1.1	Develop crop cultivars and/or germplasm with high resistance to reduce numbers of and damage from *Heliothis/Helicoverpa* spp.
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	1.1.2c	Develop transgenic plants and select new resistance genes for plant transformation
SUPPL ARRAY:	1.1.3	Develop plant resistance technology that would impact or alter the course of action of any one of the other arrays

DATES COVERED BY REPORT: September 1991-July 1993.

PROGRESS REPORT: Over the past 14 years, 292 tobacco cultivars, breeding lines, and Tobacco Introductions (1991- 68 entries, 1992- 74, 1993- 32) have been evaluated in replicated single-row field plots at Oxford, NC and Tifton, Ga. for their resistance to tobacco insect pests, including tobacco budworms, *Heliothis virescens*. Several sources of insect-resistant germplasm have been found. This material is being used in a tobacco breeding program (in cooperation with Drs. Verne Sisson, J. F. Chaplin [collaborator], and G. R. Gwynn [retired]) at the USDA-ARS, Crops Research Laboratory, Oxford, NC. A tobacco budworm-resistant tobacco breeding line (I-514) was released in 1990. This breeding line is highly attractive to tobacco budworms for oviposition, but few larvae survive past the second instar when they feed on it.

As part of a Host Plant Resistance Cooperative Project for tobacco (that includes researchers from six states), several advanced tobacco breeding lines per year (1991- 13, 1992- 13, 1993- 14) have been evaluated for insect resistance in field plots (2 replications) at Oxford, NC; Tifton, GA; Florence, SC; Greeneville, TN; Blackstone, VA, and Lexington, KY. Several tobacco budworm-resistant breeding lines have been released in conjunction with these cooperative efforts.

INVESTIGATOR'S NAME(S): J. N. Jenkins

AFFILIATION & LOCATION: USDA, ARS, CSRL, Mississippi State, MS 39762

ACTION AREA:	1.	Host Plant Resistance
LEAD ARRAY:	1.1	Develop crop cultivars and/or germplasms with high resistance to reduce numbers of and damage from *Heliothis/Helicoverpa* spp.
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DATES COVERED BY REPORT: September 1991-June 1993.

PROGRESS REPORT: Screened commercial cultivars for resistance and ranked cultivars. Evaluated day neutral race lines for resistance, but did not find any new sources. Confirmed that LA 850082 and ST 69132 germplasm contains useful levels of field resistance to *Heliothis*. ST 69132 subsequently released by commercial company as ST 132 cultivar. Compared cultivars developed between 1890 and 1986 for resistance to *tobacco budworm*. Newer cultivars yielded more and some, such as DES 119 and DPL 50 yielded as much with TBW applied as older cultivars without TBW. These newer cultivars significantly reduce the risk associated with producing cotton when high levels of TBW are present compared with most cultivars developed before 1978. Determined that LA 850082 Heliothis resistant germplasm and sprays of B.t. 1 or 2 times per week was as effective as weekly sprays of pyretheroids on commercial cultivars of cotton for control of tobacco budworm. Worked with Monsanto Agricultural Company to evaluate several lines of cotton genetically transformed to contain the gene that encodes for the delta endotoxin from *Bacillus thuringiensis*. Transgenic plants do a good job of control of tobacco budworm with slightly better control of tobacco budworm than cotton bollworm. Conducted preliminary studies on seed mixtures of B.t. transgenic and nontransgenic cotton for resistance management of delta endotoxin in transgenic plants. Completing a cooperative study with Monsanto Agricultural Company and the Agricultural Economics Department at Mississippi State University that will evaluate the effects of transgenic B.t. cotton on cotton production and economics in Mississippi. Began to develop germplasm with a combination of *Heliothis* resistance and root-knot nematode resistance.

FY94 & FY95 WORK PLANS: Continue to work with Industry to evaluate genetically transformed cotton plant for *Heliothis* control. Continue to research strategies for resistance management for genes in resistant plants. Develop and evaluate day-neutral primitive germplasm accessions for *Heliothis* resistance. Work on mechanisms of resistance and biochemical basis of resistance.

INVESTIGATOR'S NAME(S):		L. Lambert and W. R. Meredith
AFFILIATION & LOCATION:		USDA, ARS, SFCIML, Stoneville, MS 38776
ACTION AREA:	1.	Host Plant Resistance
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DATES COVERED BY REPORT: September 1991-June 1993.

PROGRESS REPORT: Cotton lines with and without nectaries were compared to determine the influence of nectar as a food source on levels of oviposition by tobacco budworm. Studies were conducted under no choice conditions in screened cages with laboratory reared insects. Results of the first year of the study showed that significantly fewer eggs were deposited, fewer larvae developed and less damage occurred on cotton without nectaries than on cotton with nectaries. However, data from the second year of the study showed no significant differences among treatments. A uncontrolled whitefly population which provided honeydew as a food source on all treatments is thought to have confounded the results the second year. Studies conducted in field cages with tobacco budworm and soybean plants with and without pubescence showed no differences in levels of parasitization due to plant pubescence type.

FY94 & FY95 WORK PLANS: Efforts will be continued to develop techniques to control aphid and other insect populations which confound study results of the influence of cotton nectar on tobacco budworm populations. Begin studies to determine the interactions of host-plant resistance in soybean with the celery looper virus in influencing cotton bollworm populations

INVESTIGATOR'S NAME(S): R. E. Lynch

AFFILIATION & LOCATION: USDA, ARS, IBPMRL, Tifton, GA

ACTION AREA:	1.	Host Plant Resistance
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DATES COVERED BY REPORT: September 1991-June 1993.

PROGRESS REPORT: A peanut core collection representative of the 7,000+ plant introductions was evaluated for resistance to insects, including *Helicoverpa zea*. Selected entries from the core collection that had reduced insect damage in previous evaluations, germplasm from Bill Campbell with resistance to insects in Thailand/Philippines, and advanced breeding lines from Bill Branch, peanut breeder at Tifton, were included in an Advanced Resistance Evaluation. These entries were rated for damage by *H. zea* in the field, and bioassayed with *H. zea* in the laboratory. Data from laboratory evaluations were expressed as a Host Suitability Index where HSI = (pupal weight/leaf consumption)/days to pupation X (% survival to adults X 100). Accessions with a high HSI are more susceptible than accessions with low HSI values. PI 179630, PI 196636, and Accession 29 (NC7 x NC343) had HSI's of 5.2, 5.6, and 5.7, respectively, while PI 196702 and PI 259829 had HSI's of 12.3 and 12.2, respectively. Segregating populations of peanut crosses made by Bill Branch from multiple pest resistant germplasm were also evaluated for insect damage. Seed from individual plants with reduced insect damage were selected for continued evaluation.

FY94 & FY95 WORK PLANS: Evaluation of the peanut core collection will be continued. As entries with a high level of resistance are identified, additional evaluations of the PI's from which the resistant germplasm originated will be conducted. Lines showing resistance to *H. zea* in the field will be evaluated for mechanism of resistance in the laboratory. Evaluation of germplasm provided by Bill Branch from crosses among pest resistant parents will continue. Cooperative research with Tom Stalker, North Carolina State University, will be initiated to evaluated crosses between the cultivated peanut, *Arachis hypogaea* and wild peanut species for resistance to insects including *H. zea*.

INVESTIGATOR'S NAME(S): R. L. Wilson

AFFILIATION & LOCATION: USDA, ARS, PIRU, Iowa State University, Ames, IA 50011

ACTION AREA:	1.	Host Plant Resistance
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DATES COVERED BY REPORT: September 1991-June 1993.

PROGRESS REPORT: In 1991, 294 PI maize accessions were evaluated for silk feeding resistance to corn earworm. In 1992, 218 accessions were evaluated. Between the two years, approximately 20 lines were identified as having a fairly high degree of resistance. All of these accessions will have to be retested to confirm the resistance. Previous evaluation of maize in the National Plant Germplasm System collection held at Ames, Iowa, identified new sources of resistance in two tropical races. The total holdings of these two races, 'Confite Puntiagudo' (21) and 'Dulcillo del Noroeste' (10) were evaluated for silk feeding resistance to *H. zea* at Ames, Iowa, Tifton, Georgia, and Hermiston, Oregon. Confite Puntiagudo is a maize race of popcorns while Dulcillo del Noroeste is a race of sweet maize. Results indicate there are sources of resistance within these races but the resistance is not stable across all environments tested. Development of locally adapted cultivars could benefit from these new sources of resistance.

In cooperation with B. R. Wiseman and M. E. Snook, the chemical nature of resistance is being determined in the laboratory. A new chemical, coined "popsin" has been identified as a chemical that suppresses corn earworm growth and development.

FY94 & FY95 WORK PLANS: I plan to continue evaluating the NPGS maize collection looking for new sources of silk feeding resistance to corn earworm. I will also evaluate corn earworm fecundity after feeding on resistant accessions. Crosses will be made to susceptible inbreds to determine the genetics of the resistance.

INVESTIGATOR'S NAME(S): B. R. Wiseman

AFFILIATION & LOCATION: USDA, ARS, IBPMRL, Tifton, GA 31793

ACTION AREA:	1.	Host Plant Resistance
LEAD ARRAY:	1.1	Develop crop cultivars and/or germplasms with high resistance to reduce numbers of and damage from *Heliothis/Helicoverpa* spp.
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DATES COVERED BY REPORT: September 1991-June 1993.

PROGRESS REPORT: Several corn lines were identified with high antibiosis : Ames 10589 (high maysin), PI340853 (high popsin), and Ames 10587 (high chlorogenic acid). Silks of A-10589 contained almost 4X the level of maysin of Zapalote Chico. Silks from recurrent selection populations ANTB-EPDS and ANTB-SIDS and 75 crosses of Zapalote chico with 11 dent inbreds were evaluated for antibiosis against corn earworm. The best 10% of plants, based on worm weights of 25 mg or less, were selected for recombination and future selection. Additional recurrent populations have been evaluated in the field for ear damage. The best performing S1 progenies (10%) were selected for recombination and future testing. In laboratory tests, silks of Zapalote Chico fed corn earworm larvae, reduced growth, extended development time by 20 d, and reduced egg production by 65%. Several commercial hybrids were identified in the laboratory as having high levels of antibiosis. A microtechnique for bioassaying individual plant parts from individual plants was perfected. A concentration of 75 mg of dry silk/ml dilute pinto bean diet produced the greatest differences between resistant and susceptible silk cultivars. An eppendorf dispenser is used to dispense the dilute diets. A combination of resistant corn silks and Elcar was shown to increase mortality of corn earworm neonates. Larvae fed 4 d on resistant silk and control diets and then exposed to Elcar resulted in as much as 98% mortality for larvae fed resistant silk-diets vs 0% for larvae exposed to no Elcar plus no resistant silks and 69% mortality for larvae exposed to Elcar plus no resistant silks. For larvae fed 8 d on resistant silk and control diets, combined resistant silks and Elcar caused 87% mortality vs 0% for larvae exposed to no Elcar plus no resistant silks and 3% for larvae exposed to Elcar plus no resistant silks.

FY94 & FY95 WORK PLANS: New corn lines and current ongoing breeding materials will be screened for new factors and/or higher levels of silk resistance. Silk bioassays will be used to determine weight differences among corn earworm larvae at 8 days. Additional tests will be made to determine the effectiveness of combining the resistant plant, IGR and/or virus combinations in the field. Initial crosses are to be made to combine high maysin, popsin and chlorogenic acid into one inbred. Silk bioassays will be used to confirm the antibiotic responses of chemical extractions. Selected commercial hybrids will be evaluated in the field for ear damage and with laboratory bioassays to confirm antibiotic responses. Corn lines with high maysin, popsin, and chlorogenic acid will be evaluated in separate tests to study their individual responses on corn earworm growth and development.

INVESTIGATOR'S NAME(S): B. R. Wiseman

AFFILIATION & LOCATION: USDA, ARS, IBPMRL, Tifton, GA 31793

ACTION AREA: 1. Host Plant Resistance

LEAD ARRAY: 1.2 Determine the biological, biochemical, and/or biophysical mechanisms of resistance to *Heliothis/Helicoverpa* spp.

SUPPL ARRAY: 1.2.3 Determine the genetic basis of the resistant plant materials and/or identified chemical(s) factors

DATES COVERED BY REPORT: September 1991-June 1993.

PROGRESS REPORT: The antibiotic mechanism of resistance was established in a number of selections from breeding materials, ANTB-SIDS, ANTB-EPDS, PI340853, Ames 10589, Ames 10587 and commercial hybrids Zimmerman Z63W and Z27, N C+ X6485, and Hyperformer HS9843. Zimmerman Z63W has no maysin. A thin-layer chromatographic method was developed for analysis of maysin and its analogues found in silks of corn resistant to the corn earworm. Three antibiotic chemicals, Maysin, popsin and chlorogenic acid, were identified and associated by correlation analysis ($r = -0.81$) with low worm weight. A concentration of $\approx 0.2\%$ maysin reduced larval growth more than 50%, and 0.4% maysin reduced weights more than 70%. Compositional differences of cuticular lipids of silks were identified from seven corn genotypes. Corn earworm neonates fed silks from first ears weighed significantly less than larvae fed silks from the second ears. Silks regrown for 1 or 2 days after initial cutting produced larger larvae at 8 days than those fed on silk-diets from the initial cutting. Relative weights of larvae and maysin content were consistent among genotypes, whether the silks were from the first or second ears. Growth of larvae was greater on silk-diets which had the cuticular lipids removed. The expression of antibiosis disappears (maysin and low worm weights) from the resistant silk by 10 d after pollination. The genetic basis of the antibiotic resistance was studied in crosses Ab18 (susceptible) X Zapalote Chico and GT114 (resistant) X GT119 (susceptible). Results indicated the additive-dominance model was unsatisfactory and the nature of the antibiotic resistance appears to be controlled by several pairs of genes. In a cross of Z. Chico X GT114, the inheritance of the antibiotic resistance involve nonadditive (dominance and epistasis) genetic variance. A digenic 6-parameter model indicated (1) the resistance in this cross is controlled by more than one pair of genes, and (2) some or all of the genes interact with each other to cause nonallelic interaction. Thus, the resistance in this cross may be controlled by dominant and recessive genes. The resistance in another cross, Z. Chico X CI64 is controlled by additive gene effects. It appears from the crosses studied that gene action differs from one type of cross to another. Bioassays comparing diets with fresh silk, and two concentrations of dry silk, and diets with maysin confirmed that the bioassay using dry silks is probably the best since corn maturities prevent bioassays of fresh silk at one time, whereas bioassays of dry silk comparing a number of corn lines with differing maturities can be bioassayed at one time. The lack of Formalin in the diets may cause erroneous results due to interactions of the diet and silk. Diets without formalin may produce small larvae, whereas the normal diet produces large larvae. Based on chemical analysis and low worm weights, silks from the top ear possess a higher maysin content and antibiotic response than silks from second ears.

FY94 & FY95 WORK PLANS: Genetic studies are planned with additional inbreds GE72, CI83A and GT114. The individual silk microbioassay will determine weight differences among larvae at 8 days. Further chemical analysis, biological determinations, and mechanisms of resistance will be made as new lines are identified with additional bases of resistance. The effects of age of silk as it relates to the expression of antibiosis will be studied among several corn genotypes. Fresh and dry silk will be fed in silk-diets. Weight of larvae at 8 days, days to pupation and weight of pupae will be recorded. Chemical analysis of fresh and dry silks will be made. Several corn populations and hybrids varying in the silk-browning response will be grown in 3 locations and the silks will be bioassayed for antibiotic responses.

TABLE 1. Summary of Research Progress for Action Area I, Host Plant Resistance, in Relation to Year 2 Goals of the 5-Year Plan.

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
1.1 Develop crop cultivars and/or germplasms with high resistance to reduce numbers of and damage from *Heliothis/ Helicoverpa* spp.	Begin initial plans to incorporate resistant germplasms into breeding and/or development program.	X		Extensive testing has identified over 20 corn accessions, 2 cotton germplasms, 3 peanut accessions, 10 species of Solanaceae and several tobacco germplasms as having *Heliothis/Helicoverpa* resistance. Three antibiotic chemicals have been identified in corn, over 60 petuniasterones and petuniolides and 10 steriod glucosides have been isolated from *Petunia* and *Physalis peruviana.* Incorporation of these resistance mechanisms into commercial crop varieties will provide useful reductions in *Heliothis/Helicoverpa* damage levels. Little progress was reported concerning the interaction of host plant resistance with other integrated pest management methods, however, the cotton germplasm LA 850082 combined with sprays of *B.t.* was as effective as sprays of pyrethriods on commercial cultivars of cotton for control of tobacco budworm. The effect of incorporating host plant resistance into IPM technology needs further research. Genetically transformed cotton encoded for the delta endotoxin of *B. t.* have been evaluated and were shown to control tobacco budworm better than cotton bollworm. Preliminary studies of seed mixtures of *B. t.* transgenic and nontransgenic cotton for resistance management have been conducted however, no results were reported. Attempts were made to introduce *H. zea* resistant genes from Petunia and Physalis to tomato and potato. Some of the putative transgenic plants are being evaluated for insect resistance. Development of transgenic plants containing resistance characters from other plant species can increase the sources of resistance available for use in commercial corp varieties.

TABLE 1 - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
1.2 Determine the biological, biochemical, and/or biophysical mechanisms of resistance to *Heliothis/Helicoverpa* spp.	Conduct laboratory and/or field studies to determine effects and interactions of resisted plant materials on the pest insect.	X		A microtechnique for bioassaying individual plant parts such as silks was perfected and resistant silks increased mortality of corn earworm by Elcar by as much as 90%. The antibiotic mechanism of resistance was established for several corn lines and commercial hybrids. Three antibiotic chemicals (maysin, popsin and chlorogenic acid) were identified and associated by correlation analysis with low worm weight. The expression of antibiosis disappeared from resistant silks by 10 d after pollination. Studies of genetic basis of antibiotic resistance indicated that resistance was controlled by several pairs of genes. Several species from the *Solanaceae* family of plants were found to have significant resistance to *H. zea* and attempts were made to introduce resistance genes from *Petunia* and *Physalis* to tomato and potato by protoplast fusion. Some of the putative transgenic plants are being evaluated for insect resistance. Over sixty petuniasterones and petuniolides were isolated from several *H. zea* resistant species of *Petunia* and their chemical structures determined. Similarly, ten steroid glycosides have been isolated and identified from *Physalis peruviana.* At levels as low as 2.5 ppm, these compounds have been shown to reduce the larval growth of *H. zea* to one-half that of larvae grown on control diet. Identification of resistance characters and chemicals associated with antibiosis can ultimately play a role in development of resistant commercial varieties of plants.

RESEARCH SUMMARY: ACTION AREA I—HOST PLANT RESISTANCE

Compiled by Lavone Lambert and Billy Wiseman

LEAD ARRAY 1.1: At Tifton, Georgia, numerous corn accessions and current breeding lines were evaluated annually for resistance to corn earworm. Ames 10589 (high maysin); PI340853 (no maysin but high popsin), and Ames 10587 (high chlorogenic acid) have been identified as resistant. Silks of A-10589 contained almost 4X the level of maysin found in Zapalote Chico. Plants from recurrent selection populations ANTB-EPDS and ANTB-SIDS were evaluated for high antibiosis against the corn earworm. The best 10% of the plants were selected for recombination and future selection. Crosses of Zapalote Chico with 11 dent inbreds were each evaluated in the laboratory and field for high antibiosis and the best 10% were selected for recombination and future evaluation. Silks of Zapalote Chico fed corn earworm larvae, reduced growth, extended development time by 20 d, and reduced egg production by 65%. Several commercial hybrids have been identified in the laboratory as having high levels of antibiosis against corn earworm larvae.

At Ames, Iowa, 512 corn accessions were evaluated for silk feeding resistance to corn earworm. In 1992, 218 accessions were evaluated of which 20 were identified as having resistance and are to be retested to confirm the resistance. New sources of resistance to *H. zea* were found in two tropical races, 'Confite Puntiagudo' (21) and 'Dulcillo del Noroeste' (10). Results indicate the sources of resistance within these races is not stable across all environments. In cooperation with B. R. Wiseman and M. E. Snook, the chemical nature of resistance is being determined in the laboratory. A new chemical, coined "popsin" has been identified as a chemical that suppresses corn earworm growth and development.

At Mississippi State, Mississippi, commercial cotton cultivars were evaluated with day neutral race lines for resistance to *H.* spp. No day natural lines were resistant but confirmed that LA 850082 and ST 69132 germplasm contains useful levels of field resistance to *H.* spp. ST 69132 has been released by a commercial company as ST 132 cultivar. Cultivars developed between 1890 and 1986 were compared for resistance to tobacco budworm. Newer cultivars yielded more, and some, such as DES 119 and DPL 50 yielded as much with TBW applied as older cultivars without TBW. LA 850082 Heliothis resistant germplasm combined with *B. t.* sprays was as effective as pyrethriod sprays on commercial cultivars of cotton for controlling tobacco budworm. Several lines of cotton genetically transformed to contain the gene that encodes for the delta endotoxin from *B.t.* were evaluated. Transgenic plants do a good job of control of *H.* spp. with slightly better control of tobacco budworm than cotton bollworm. Preliminary studies were conducted on seed mixtures of B.t. transgenic and nontransgenic cotton for resistance management of delta endotoxin in transgenic plants. Evaluated the effects of transgenic *B.t.* cotton on cotton production and economics in Mississippi. Development of germplasm with a combination of *Heliothis* resistance and root-knot nematode resistance is underway.

At Stoneville, Mississippi cotton lines with and without nectaries were compared to determine the influence of nectar as a food source on levels of oviposition by tobacco budworm under no choice conditions. Results of the first year of the study showed that significantly fewer eggs were deposited, fewer larvae developed and less damage occurred on cotton without nectaries than on cotton with nectaries. However, data from the second year of the study showed no significant differences among treatments. An uncontrolled whitefly population which provided honeydew as a food substrate on all treatments is thought to have confounded the results the second year. Studies conducted in field cages with tobacco budworm and soybean plants with or without pubescence showed no differences in levels of parasitization due to plant pubescence type.

At Tifton, Georgia a representative peanut core collection was evaluated for resistance to *Helicoverpa zea.* Selected entries from the core collection that had reduced insect damage in previous evaluations were included in an Advanced Resistance Evaluation. PI's 179630, and 196636, and Accession 29 (NC7 x NC343) had Host Suitability Indexes (HSI's) of 5.2, 5.6, and 5.7, respectively, while PI's 196702 and 259829 had HSI's of 12.3 and 12.2, respectively.

At Oxford, North Carolina tobacco cultivars, breeding lines, and Tobacco Introductions have been evaluated for resistance to tobacco budworms. Several sources of insect-resistant germplasm have been found which are being utilized in tobacco breeding program to develop resistance. A tobacco budworm-resistant breeding line (I-514)

was released in 1990. This breeding line is highly attractive to tobacco budworms for oviposition, but few larvae survive past the second instar when they feed on it.

As part of a Host Plant Resistance Cooperative Project for tobacco that includes six states, 13-14 advanced breeding lines per year have been evaluated for insect resistance. Several tobacco budworm-resistant breeding lines have been released in conjunction with these cooperative efforts.

LEAD ARRAY 1.2: At Tifton, Georgia, a microtechnique for bioassaying individual plant parts such as silks from individual plants has been perfected. Resistant silks increase the mortality of corn earworm by Elcar by as much as 90%. The antibiotic mechanism of resistance has been established for ANTB-SIDS, ANTB-EPDS, PI340853, Ames 10589, Ames 10587 and commercial hybrids : Zimmerman Z63W and Z27, N C+ X6485, and Hyperformer HS9843. Three antibiotic chemicals have been identified and associated by correlation analysis ($r = -0.81$) with low worm weight : Maysin, popsin and chlorogenic acid. The expression of antibiosis disappears (maysin and low worm weights) from the resistant silk by 10 d after pollination. The genetic basis of the antibiotic resistance was studied in crosses Ab18 (susceptible) X Zapalote Chico and GT114 (resistant) X GT119 (susceptible). Results indicated that the additive-dominance model was unsatisfactory and that the nature of the antibiotic resistance appears to be controlled by several pairs of genes.

At Albany, California 10 species from the *Solanaceae* family of plants were found to have significant resistance to *H. zea*. Attempts were made to introduce *H. zea* resistant genes from *Petunia* and *Physalis* to tomato and potato by protoplast fusion. Some of the putative transgenic plants are being evaluated for insect resistance. Over sixty petuniasterones and petuniolides were isolated from several *H. zea* resistant species of *Petunia* and their chemical structures determined. Similarly, ten steroid glycosides have been isolated and identified from *Physalis peruviana*. At levels as low as 2.5 ppm, these compounds have been shown to reduce the larval growth of *H. zea* to one-half that of larvae grown on control diet. The exact nature of the resistance mechanism is unknown.

BREAKOUT SESSION SUMMARY

The HPR group agreed that plant resistance should be a main component in area-wide suppression of *Heliothis/Helicoverpa*. Plant resistance and transgenic plants could be main factors in reducing populations and this technology will easily integrate with other control measures. With the reality and proliferation of transgenic plants, researchers in HPR should be instrumental in their evaluation. It was noted that many HPR traits are currently "on the shelf", and a marketing strategy should be developed to use them. The group considered that evaluation of existing corn, cotton tobacco, soybean, and sorghum germplasm for resistance was a priority. Resistance traits within the same species would be desirable, however, resistance sources within closely related species from the area of origin of a particular crop should be considered. They considered that a high degree of resistance would be desirable, but measurable resistance would be useful. The group further agreed that compatibility of HPR with other methods of control, particularly biological control, should be demonstrated. The group further noted that resistance traits are often not used because of the availability of effective insecticides, frequent lower yields associated with the resistance traits, inadequate communications between seed producers and researchers, frequent lack of cooperation among scientists, and lack of profitability to seed producers if resistant cultivars are available to all seed producers. They felt that patenting and licensing of germplasm sources may alleviate part of this problem.

Action Area II. Chemical Control and Application Technology

Coordinators: I. W. Kirk and D. A. Wolfenbarger

INVESTIGATOR'S NAME(S):		L. D. Chandler
AFFILIATION & LOCATION:		USDA, ARS, IBPMRL, Tifton, GA 31793
ACTION AREA:	2.	Chemical Control and Application Technology
LEAD ARRAY:	2.1	Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean)
SAFEGD ARRAY:	2.1.1	Identify new insecticides/biorationals and/or application techniques for optimization of Lead Arrays 2.1 and 2.2
OPTIM ARRAY:	2.1.2a	Develop improved formulations of candidate insecticides/biorationals
SUPPL ARRAY:	2.1.3a	Conduct studies to determine environmental fate of best candidate insecticides/biorationals using various application systems
SUPPL ARRAY:	2.1.3c	Conduct studies to develop insecticide resistance management strategies for *Heliothis/Helicoverpa*

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: The biorational insecticides ***B. thuringiensis***, azadirachtin, M- PEDE, and fenoxycarb provided high levels of *H. zea* mortality; however, cotton flour based feeding stimulants did not improve efficacy. Thiodicarb, esfenvalerate, cypermethrin, chlorpyrifos, and bifenthrin were effective against *H. zea* larvae at labeled rates. Thiodicarb was least harmful to predatory insects. Cypermethrin, chlorpyrifos, and bifenthrin provided > 80% control at less than half the lowest labeled rates. Sublethal rates of diflubenzuron on *H. zea* larvae caused various levels of adult sterility. Low rates of chlorpyrifos plus cypermethrin improved control of *H. zea* compared to either insecticide alone as determined by analysis of deviance. Non-EC petroleum oil added to chemigated cypermethrin, bifenthrin, esfenvalerate, and chlorpyrifos increased bollworm mortality and residual activity in cotton. Addition of oil to ***B. thuringiensis*** resulted in poorer bollworm mortality than when applied without oil. Chemigation of cypermethrin plus chlorpyrifos using rotating sprinklers on drop tubes provided better control of larvae in fresh market corn than conventional application or chemigation with impact sprinklers. Application of NPV (Elcar) via chemigation reduced *H.zea* moth production in field corn. An experimental IGR, RH-5992, was effective against bollworm and fall armyworm larvae. A new entomogenous nematode, *Steinernema riobravis*, was evaluated for control of bollworm larvae in whorl and silk stage corn with varying success. Studies were developed to determine the fate of chlorpyrifos and esfenvalerate applied to corn following heavy rainfall. To date little chemical residue has been found in plant washoff or field runoff.

FY94 & FY95 WORK PLANS: Laboratory and field studies to identify new biorational and chemical insecticides to manage bollworm on corn, cotton and peanuts will continue. Experiments will compare interactions between biological and chemical control methods and determine ability to integrate insecticides into crop management programs emphasizing reduced chemical insecticide use. Tests will continue to determine the feasibility of using NPV in chemigation for early season bollworm management on corn. Evaluation of *S. riobravis* as a foliar and soil insecticide applied via chemigation in corn will continue. Experiments will evaluate chemigation vs. conventional application methods in corn, cotton and peanuts with emphasis on use of biorational insecticides and reduced rates of chemical insecticides. Studies to determine field runoff of pyrethroid insecticides from cotton will be initiated.

INVESTIGATOR'S NAME(S): G. W. Elzen

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS 38776

ACTION AREA:	2:	Chemical Control and Application Technology
LEAD ARRAY:	2.1	Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)
SAFEGD ARRAY:	2.1.1	Identify new insecticides/biorationals and/or application techniques for optimization of Lead Arrays 2.1 and 2.2
SUPPL ARRAY:	2.1.3b	Conduct studies to develop a theory of inheritance of insecticide resistance and to determine insecticide resistance mechanisms
SUPPL ARRAY:	2.1.3c	Conduct studies to develop insecticide resistance management strategies for *Heliothis/Helicoverpa*

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Insecticides and biologicals were evaluated in small plots and in the laboratory using several bioassays. Registered, conjugated *B. t.*'s were tested. Documented increased resistance levels in tobacco budworm to endosulfan and found that this resistance was not synergizable. The responses of an insecticide resistant strain of *Heliothis virescens* were examined in the laboratory using two bioassays during continuous culture without insecticide selection pressure. Resistance to the pyrethroid cypermethrin and the carbamate thiodicarb did not revert to susceptibility until 12 generations in culture. The temporal sequence of resistance in field collected *H. virescens* in 1992 was examined using the adult vial test and the spray table bioassay. Resistance to four classes of insecticides was variable but often at high levels prior to and during the cotton growing season. Topical bioassays of cypermethrin, endosulfan, methomyl, profenofos, and sulprofos were conducted on colonies from Louisiana, Mississippi, and Texas. The field collected colonies exhibited low to high levels of resistance to cypermethrin (1-42x), low to moderate levels of resistance to profenofos and sulprofos (1-6x), and low to high levels of resistance to methomyl (2-21x). Spray chamber bioassays indicated reduced efficacy of cypermethrin, endosulfan, profenofos, and thiodicarb against the field collected colonies of tobacco budworms. The adult vial test (AVT) was evaluated for utility in predicting resistance levels in tobacco budworm to non-pyrethroid insecticides and in predicting resistance to OP's and pyrethroids in the boll weevil. Field strains collected through the season in Washington County, Miss., were used to establish baseline discriminating doses for the AVT. The presence of metabolic resistance was detected using inhibitors of mixed function oxidases and hydrolytic esterases.

FY94 & FY95 WORK PLANS: To evaluate the presence of cross-resistance patterns in *H. virescens*. To evaluate resistance by *H. virescens* to endosulfan. Inheritance of resistance to endosulfan in *H. armigera* is sex linked. Cross-resistance to other cyclodienes (dieldrin, aldrin) and inheritance of resistance to cyclodienes in *H. virescens* will be evaluated. To evaluate additivity or synergism of mixtures of insecticides applied for control of *tobacco budworm* and to evaluate the effect of low and high dose mixtures of *B. t.*'s with conventional insecticides. To continue to monitor resistance levels in *Heliothis* using a variety of insecticide bioassays. Resistance management plans depend on current information regarding trends in the development of resistance in field populations.

INVESTIGATOR'S NAME(S): D. M. Jackson

AFFILIATION & LOCATION: USDA, ARS, CRL, Oxford, NC

ACTION AREA: 2: Chemical Control and Application Technology

LEAD ARRAY: 2.1: Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)

SAFEGD ARRAY: 2.1.1: Identify new insecticides/biorationals and/or application techniques for optimization of Lead Arrays 2.1 and 2.2

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: A synthetic pyrethroid insecticide, cyhalothrin, was tested in the field for control of tobacco budworm larvae in tobacco seed nurseries. This material was highly effective when applied both by a backpack sprayer and by a high-boy, tractor-mounted sprayer. A special use permit for cyhalothrin to control budworms on state experiment stations was sought.

Ten treatment combinations containing MVP (a *Bacillus thuringiensis* product from Mycogen Corporation) and other formulations of *Bacillus thuringiensis* were tested for efficacy against tobacco hornworm and tobacco budworm larvae on flue-cured tobacco in the field. Some of the new formulations were quite effective in controlling *H. virescens* larvae.

INVESTIGATOR'S NAME(S): I. W. Kirk and L. F. Bouse

AFFILIATION & LOCATION: USDA, ARS, SCRL, AARU ,College Station, TX

ACTION AREA: 2: Chemical Control and Application Technology

LEAD ARRAY: 2.1: Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean)

OPTIM ARRAY: 2.1.2b: Develop improved insecticide application methodology for creation of optimal droplet sizes, reduction in drift, and increased probability of deposition onto the target

LEAD ARRAY: 2.2: Determine and compare optimal insecticide application techniques utilizing best available technology (aerial, ground-rig, chemigation, etc.) to improve application methods for improved efficacy and lower environmental impact of currently used chemical insecticides and biorationals

OPTIM ARRAY: 2.2.2: Develop improved insecticide application methodology for creation of optimal droplet sizes, reduction in drift, and increased probability of deposition onto the target

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Pesticide formulation, mixture concentration, surfactants, polymers, spray pressure, nozzle type and orientation were all determined to effect spray droplet size for pressure nozzles operating in an airstream. A simple method was developed for demonstrating techniques for reducing spray drift. The relationship between droplet size and drift reduction techniques provide guidance in selecting and operating aerial spray equipment for optimizing aerial application practices. Effects of droplet size, droplet density, spray rate, and active ingredient rate on *Heliothis* egg and larval mortalities were determined for three ovicides in laboratory, greenhouse and aerial field scale studies on cotton. Active ingredient rate is a dominant factor in ovo-larvicidal efficacy. Spray droplet spectra and spray rate must be optimized for each ovicide for maximum efficacy.

Effects of droplet spectra on *Heliothis* larval mortalities were determined for four synthetic pyrethroid insecticides in laboratory/greenhouse studies. Droplet size was more important in efficacy for some pyrethroids than for others. These studies show that efficacy of the four pyrethroids can be maintained when droplet size is increased by switching aerial nozzle orientation from down to back, as is often done to reduce drift to sensitive environments. Relationships between aerial spray rate, droplet size, droplet density, temperature, relative humidity, wind speed and active spray ingredient deposition in cotton canopies were determined. Droplet spectra and spray rate can be modified by the applicator to improve deposition and distribution in broadleaf row crop canopies. Adverse environmental factors may be avoided by spray applicators to maintain deposition efficiency.

These studies show that efficacy can be increased at standard and lower active ingredient rates by optimizing application parameters.

FY94 & FY95 WORK PLANS: Determine the effects of combinations of application parameters on *Heliothis* egg and larval mortalities with ovicides and pyrethroids that have not been possible with current laboratory spray systems. (A spray chamber with extended capabilities is being developed for these studies.) These studies will be expanded to include biological and natural product insecticides. Field studies will be conducted to determine the effect of optimized application parameters on efficacy and drift.

INVESTIGATOR'S NAME(S): M. A. Latheef and O. D. Dailey, Jr.

AFFILIATION & LOCATION: USDA, ARS, SCRL, AARU, College Station, TX, and USDA, ARS, SRRC, New Orleans, LA

ACTION AREA: 2: Chemical Control and Application Technology

LEAD ARRAY: 2.1: Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)

SAFEGD ARRAY: 2.1.1: Identify new insecticides/biorationals and/or application techniques for optimization of Lead Arrays 2.1 and 2.2

OPTIM ARRAY: 2.1.2a: Develop improved formulations of candidate insecticides/biorationals

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Polymeric controlled release formulations of sulprofos were compared with an emulsifiable concentrate (EC) formulation of sulprofos for efficacy against the tobacco budworm (TBW), *Heliothis virescens* eggs and larvae on cotton. The ethyl cellulose formulation of sulprofos caused significantly greater mortality of TBW larvae (avg 74%) than the other polymeric formulations (avg 48%); but there was no significant difference in mortality of larvae between sulprofos EC and the ethyl cellulose formulation of sulprofos. The poly(_-methylstyrene) formulation of sulprofos exhibited significantly greater ovicidal mortality of TBW than the sulprofos EC 5 days after treatment (28.7 versus 1.8%). Ovicidal mortality caused by sulprofos EC averaged 4.8%. Overall, polymeric capsules slightly increased ovicidal mortality of sulprofos (6.1 to 14.1%), but not significantly so.

The ß-cyclodextrin (BCD) complex of sulprofos was prepared in an aqueous medium. The BCD complexes were characterized by solubility properties, elemental analyses, and spectral studies (ultraviolet, infrared, proton NMR, and ^{13}C NMR spectroscopy), which provided evidence confirming the formation of the true inclusion complex. Sulprofos EC caused significantly greater mortality of TBW larvae than a ß-cyclodextrin complex of sulprofos (83 versus 60%). Addition of xanthan gum as a suspending agent to the BCD complex significantly increased larval mortality (71 versus 60%). However, this increased mortality was significantly less than that caused by sulprofos EC (71 versus 83%).

Encapsulation of profenofos did not significantly improve its efficacy against TBW. Neither active ingredient rate (0.28 and 0.56 kg/ha) nor formulations (encapsulated profenofos, profenofos EC and thiodicarb 3.2F) significantly influenced TBW mortality on cotton. Irrespective of formulations, a spray rate of 21.5 liter per ha/large droplet combination produced a greater TBW mortality than a spray rate of 10.5 liter per ha/small droplet combination.

FY94 & FY95 WORK PLANS: Determine the physical and chemical characteristics of pest control materials to maximize efficacy against the tobacco budworm on cotton.

INVESTIGATOR'S NAME(S): J. E. Mulrooney and A. R. Womac

AFFILIATION & LOCATION: USDA, ARS, SFCIML, ATRU, Stoneville, MS

ACTION AREA: 2: Chemical Control and Application Technology

LEAD ARRAY: 2.2: Determine and compare optimal insecticide application techniques utilizing best available technology (aerial, ground-rig, chemigation, etc.) to improve application methods for improved efficacy and lower environmental impact of currently used chemical insecticides and biorationals

OPTIM ARRAY: 2.2.2: Develop improved insecticide application methodology for creation of optimal droplet sizes, reduction in drift, and increased probability of deposition onto the target

SUPPL ARRAY: 2.2.3: Elucidate mechanisms of insecticide transfer from plant surface to insect (persistence of insecticide on plant)

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: The transfer of bifenthrin/carrier combinations from cotton leaves and inert surfaces (glass and wax) showed that oil carriers transferred significantly greater (up to 50%) amounts of bifenthrin from cotton leaves to tobacco budworm (TBW) than water. The transfer of bifenthrin using vegetable and hydrocarbon based oils from cotton leaves to larvae was affected by carrier viscosity. Feeding tests showed that pyrethroid-resistant TBW were more tolerant to gossypol than susceptible TBW. Differences in performance of TBW in feeding bioassays were attributed to differences in the amount of time since outcrossing to the wild. Spray equipment investigations indicated that several high-volume air-assisted sprayers provided the greatest spray coverage on the underside of cotton leaves. Increased spray rate and level of air assistance increased canopy penetration and spray deposition. Sprays of bifenthrin and parafinic oil mixtures were applied on cotton leaves to investigate droplet size effects on the mortality of TBW. Mortality was significantly affected by bifenthrin rate, although droplet size did not result in significant mortality differences at 117 HAT. Bifenthrin applied in 337 μm vmd droplets caused larvae to die in 13% less time than the treatment in 96 μm vmd droplets. Deposition of bifenthrin on cotton in aerial spray tests was increased by the use of Chimavir winglets mounted to the spray boom. Decreased deposition of bifenthrin was observed on cotton planted in 30" rows as compared to 40" rows. In air-assisted sprayer tests, increased spray rate predominantly increased deposition and chemical efficiency under most conditions. Sprays assisted with air (velocities up to 16 m/s) increased fluorescent tracer deposits on the canopy middle up to 92% of that from the canopy top, and resulted in significant increase in bifenthrin on leaves and squares located within the canopy. Downwind spray drift resulting from aerial application was investigated using high-volume air samplers in a cotton field. Drift from a turbine aircraft traveling at 218 km/h was significantly greater than at 241 and 265 km/h. Drift from a spray boom equipped with Chimavir winglets was significantly less at a release height of 3.0 m as compared to release heights of 4.6 m and 6.1 m.

FY94 & FY95 WORK PLANS: Investigation of insect/host plant/insecticide interactions will be continued as will research on spray drift and deposition from aircraft and air-assisted ground sprayers.

INVESTIGATOR'S NAME(S): W. P. Scott

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 2: Chemical Control and Application Technology

LEAD ARRAY: 2.1: Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)

OPTIM ARRAY: 2.1.2b: Develop improved insecticide application methodology for creation of optimal droplet sizes, reduction in drift, and increased probability of deposition onto the target

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Large field tests demonstrated that deltamethrin applied at 0.02 and 0.016 lb [AI]/acre was as effective as cyfluthrin and cyhalothrin applied at 0.033 lb[AI]/acre in controlling field populations of bollworm/tobacco budworm.

Large field studies indicated in 1992 that higher populations of bollworm/tobacco budworm occurred in 30" than 40" row cotton.

Spray table test demonstrated that when petroleum diluents were mixed with bifenthrin increased mortality was not observed above the bifenthrin plus water on susceptible or resistant tobacco budworm larvae. Tests were conducted to measure deposition of bifenthrin in the canopy of 30" and 40" row spacings of cotton. Overall mean deposition was significantly higher in the 40" cotton. (Year 1)

Sprays of bifenthrin and paraffimic oil mixtures were applied to cotton leaves to investigate droplet size effects on the mortality of tobacco budworm. Results demonstrated that bifenthrin applied in 337 μm droplets caused larvae to die in 13% less time than the treatment in 96 μm droplets.

FY94 & FY95 WORK PLANS: Continue large field test comparing deltamethrin to other available pyrethroids. Expand tests to measure deposition of bifenthrin in 30" and 40" cotton.

INVESTIGATOR'S NAME(S): H. R. Sumner

AFFILIATION & LOCATION: USDA, ARS, IBPMRL, Tifton, GA

ACTION AREA: 2. Chemical Control and Application Technology

LEAD ARRAY: 2.1 Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)

SAFEGD ARRAY: 2.1.1 Identify new insecticides/biorationals and/or application techniques for optimization of Lead Arrays 2.1 and 2.2

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: The effect of oil volume and chemical rate on fall armyworm control was evaluated on whorl stage corn. Lorsban mixed with oil and applied with spray nozzles (50 gal/A) had a chemical rate response between 1/16 to 1/2 lb AI/A, but here was no improved larval control for oil up to 4 L/A. A center pivot attached sprayer system (PASS) that applied 250 gal/A of spray mixture was developed using micro-irrigation sprinklers and drip irrigation components. It is ready for evaluation as a low cost PASS for applying insecticides.

The velocity of water flow within a chemigation water line at the injection port significantly influenced the size distribution of immiscible oil droplets in the system. Three insecticide/oil droplet size distributions represented by volume median diameters of 6, 37, and 74 microns (small, medium, and large) were established to evaluate droplet size effect on larval control. When small, medium, or large droplets of mixtures of chlorpyrifos and immiscible oil were applied through a laboratory-simulated irrigation system onto corn and cotton plants that were infested with fall armyworm (FAW) larvae, the small droplets were less effective in controlling FAW than were the large droplets. The percent larval control reached 90.4% and 83.6% on corn and cotton, respectively, at half the low recommended insecticide dose. The results confirmed that chemigation is an effective application method for FAW control.

FY94 & FY95 WORK PLANS: Laboratory and field studies will be continued to determine the effect of droplet size distribution, chemical rate, water volume, and formulation on bollworm control on cotton and corn. Methods will be developed and evaluated to apply insecticides and biorationals that improve efficiency and reduce potential drift problems. Chemigation and irrigation center pivot attached sprayer will be evaluated and compared with conventional application systems. A shielded nozzle drop sprayer will be further developed and evaluated on cotton for applying bollworm control agents.

INVESTIGATOR'S NAME(S): P. V. Vail, T. H. Henneberry and M. R. Bell

AFFILIATION & LOCATION: USDA, ARS, HCRL, Fresno, CA; USDA, ARS, WCRL, Phoenix, AZ; and USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 2. Chemical Control and Application Technology

LEAD ARRAY: 2.1 Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)

SAFEGD ARRAY: 2.1.1 Identify new insecticides/biorationals and/or application techniques for optimization of Lead Arrays 2.1 and 2.2

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: The nuclear polyhedrosis virus, Af*M*NPV, has a broad host range and is infectious to *H. zea, H. virescens, Spodoptera exigua* and *Trichoplusia ni*. Susceptibility of *Pectinophora gossypiella* was demonstrated. In 1991 small field tests showed *H. zea* and *H. virescens* were approximately equal in susceptibility to residues of Af*M*NPV on cotton leaves. By day 7 less than 50% of the original activity towards *H. zea* was left on leaves when 4×10^{13} PIB/acre were applied. Residues of 4×10^{12} and 4×10^{11} PIB/acre caused less than 50% mortality 3 days after application. In 1991 similar field tests were conducted to compare Af*M*NPV and the *Heliothis* virus. Only *H. zea* larvae were used in these tests as they are slightly less susceptible to Af*M*NPV. Responses to the viruses were similar; less than 50% of the activity on leaves was left after 7 days for all dosages tested. Results of these studies showed that Af*M*NPV has potential as a microbial control agent for control of both species.

In 1992 small field trials were conducted at all locations to further elucidate the potential of Af*M*NPV. Insects included *Helicoverpa zea, Heliothis virescens, Spodoptera exigua* and *Trichoplusia ni*. Tests were conducted with a fluorescent brightener (M2R) to determine if field persistence increased. Time to 50% loss of original was extended from 5.5 to 11.5 days at the high Af*M*NPV rate with M2R. Tests in Arizona confirmed that all four species could be infected under field conditions. At Stoneville, MS, the addition of M2R had no influence on field performance of Af*M*NPV. However, the concentration was probably too low. Af*M*NPV infectivity to *H. virescens* larvae was equal to or greater than nuclear polyhedrosis viruses isolated from the alfalfa looper and *Heliothis*. The addition of COAX in the Stoneville tests did not provide a clear pattern as to the relative merits of this adjuvant. Among the three locations the field infectivity of Af*M*NPV to the four species was demonstrated. During the period laboratory studies were conducted on potential enhancement of Af*M*NPV by M2R. Increases in Af*M*NPV activity of 7.8, 4.3, 2.9, and 13.6 fold were obtained when M2R was fed with Af*M*NPV to *T. ni, H. virescens, H. zea* and *S. exigua*, respectively. Concentrations from 0.25 to 1% provide the enhancement effect with *T. ni* larvae.

FY94 & FY95 WORK PLANS: Determine M2R concentrations required in field applications to provide enhancement effects observed in laboratory; determine persistence of Af*M*NPV and M2R under field conditions; determine infectivity of Af*M*NPV formulation; conduct laboratory and field persistence tests with formulations developed specifically for Af*M*NPV.

INVESTIGATOR'S NAME(S): D. T. Wicklow, P. F. Dowd, and J. B. Gloer

AFFILIATION & LOCATION: USDA, ARS, NCAUR, Peoria, IL, and University of Iowa, Iowa City, IA

ACTION AREA: 2. Chemical Control and Application Technology

LEAD ARRAY: 2.1 Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)

SAFEGD ARRAY: 2.1.1 Identify new insecticides/biorationals and/or application techniques for optimization of Lead Arrays 2.1 and 2.2

OPTIM ARRAY: 2.1.2a Develop improved formulations of candidate insecticides/biorationals

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: A number of fungi produce sclerotia, long-lived, resting structures, that enable the fungus to survive in the absence of a living host, or to endure unsuitable environments. *Claviceps purpurea* produces a sclerotium that has been exploited for pharmaceutically important ergot alkaloids. We theorized that fungal sclerotia, like the seeds of many vascular plants, should have superior chemical defenses to prevent losses to fungal-feeding insects. This offered the first ecological explanation for the presence of potent bioactive metabolites in ergot sclerotia that are not produced by the vegetative mycelium. Sclerotia form on plant/insect hosts or their remains, and may also be produced through solid substrate fermentation. Sclerotia rarely form in liquid shaken culture, which historically has been the industry standard for producing fungal mycelium and fermentation broths, to be extracted for their primary screens. There is no reason to believe that production of bioactive metabolites is limited to *Claviceps* sclerotia.

We observed that sclerotia of *Aspergillus flavus* are avoided by the common detritivorous beetle *Carpophilus hemipterus*, an insect that feeds on the conidia and mycelia of the same fungus. Dihydroxyaflavine, was isolated and found to be a potent antiinsectan sclerotial metabolite when incorporated into a pinto bean diet at 100 p.p.m. D.W. Naturally occurring levels of this metabolite are higher in *A. flavus* sclerotia. Disydroxyaflavinine proved non-toxic to vertebrates at 300 mg/kg. General studies of the chemistry of *Aspergillus* sclerotia have led to the isolation of new antiinsectan natural products, including several compounds with oral activity against *Helicoverpa zea* comparable to that of malathion and permethrin. Results to date, have led to the discovery of over seventy natural products; forty-five possess previously unreported chemical structures including eight new ring systems. A few of these compounds and primary sclerotial extracts have been subjected to biological evaluation.

FY94 & FY95 WORK PLANS: Conduct laboratory studies to further identify and evaluate antiinsectan natural products with emphasis on sclerotial metabolites of *Aspergillus* spp. Purify and test potent antiinsectans and determine effects of best new compounds on *Heliothis/Helicoverpa* and non-target organisms.

INVESTIGATOR'S NAME(S): D. A. Wolfenbarger

AFFILIATION & LOCATION: USDA, ARS, SARL, CIRU, Weslaco, TX

ACTION AREA: 2. Chemical Control and Application Technology

LEAD ARRAY: 2.1 Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGRs, feeding stimulants and attractants) and determine optimal management strategies for *Heliothis/Helicoverpa* on important agronomic crops (corn, cotton, peanuts, soybean, etc.)

SUPPL ARRAY: 2.1.3b Conduct studies to develop a theory of inheritance of insecticide resistance and to determine insecticide resistance mechanisms

SUPPL ARRAY: 2.1.3c Conduct studies to develop insecticide resistance management strategies for *Heliothis/Helicoverpa*

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Bollworm, *Helicoverpa zea*/tobacco budworm, *Heliothis virescens* populations were susceptible to sprays of cypermethrin at 0.066 to 0.088 kg (a.i.)/ha in the Brazos Valley in 1989-1990..No relationship was observed between mortalities of moths of the tobacco budworm by the vial bioassay and percentage control in the field plot 2 to 6 days after the spray applications. Vial bioassays ranged from > 1 μg/vial at the beginning of the season in June to 26 (1990) to 50 (1989) μg/vial at the end of the season in September.

Larvae of tobacco budworm *Heliothis virescens* from the Lower Rio Grande Valley of Mexico were susceptible to cypermethrin in 1991 and 1992. In 1992 LD_{50}'s for cypermethrin against the tobacco budworm collected from cotton in the Lower Rio Grande Valley ranged from a high of 0.68 μg/larva to a low of 0.0038 μg/larva. The high LC50 value is greater than any previously determined in the Lower Rio Grande Valley. In 1992 we determined that the LD_{50} levels for methyl parathion in a tobacco budworm population was linked to the male.

FY94 & FY95 WORK PLANS: Monitoring for resistance to deltamethrin by tobacco budworm from "La Laguna" Torreon area will continue. Higher LD50's to deltamethrin were observed there compared to those from the Lower Rio Grande Valley of Mexico or the United States. We wanted to determine if resistance to deltamethrin was still present in tobacco budworms from Torreon.

Monitoring for resistance in bollworm (corn earworm) in the Mante-Tampico area of Tamaulipas, Mexico will be initiated. A high LD50 was observed in this area 10 years ago. A colony was obtained in 1993 from that same area to determine if that same resistance level was still present.

Selection for resistance in a field collected strain of tobacco budworm to cypermethrin, zetacypermethrin, alphamethrin, cythalothrin and Lambda cyhalothrin will continue. LC50 values from a non-selected field strain and a laboratory strain will be compared to the selected strain. Objective is to determine if resistance develops faster in resolved isomers of multiisomer cypermethrin and cyhalothrin.

TABLE 2. Summary of Research Progress for Action Area II, Chemical Control and Application Technology, in Relation to Year 2 Goals of the 5-Year Plan.

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
2.1 Identify effective commercially available chemical insecticides and biorationals (including biologicals, IGR's, feeding stimulants and attractants) and determine optimal management strategies for Heliothis/Helicoverpa on important agronomic crops (corn, cotton, peanuts, soybean, etc.).	Select best candidate compounds from both chemical insecticide and biorational groups for further lab and field testing. Begin screening for resistance to the best candidate insecticides and determine effects of candidate insecticides on non-tartet organisms (parasites, predators, aquatic fauna, etc.	X		Numerous commercially available chemical and biorational insecticides as well as various formulations were evaluated in laboratory and field experiments for control of *Heliothis/Helcoverpa* eggs and larvae in cotton, corn and tobacco. Most of the compounds are highly efficacous at labeled rates. Some of the synthetic pyrethriods gave >80% control at less than half of the lowest labeled rates. Combinations of some insecticides at less than recommended label rates provided better control than recommended rates of either chemical alone. Feeding stimulants did not increase mortality with biorationals. Experimental compounds including IGR's, new varieties of B. t. new isolates of Aspergillus spp. and nuclear polyhedrosis virus were shown to have potential as control agents and warrant further research and development. Monitoring of tobacco budworm insecticide resistance indicated variable but often high levels of resistance. No relationship was found between adult vial mortalities and control of tobacco budworm in field studies. Methods were developed to determine the fate of synthetic pyrethroids following rainfall. No research was reported on the theory of inheritance of insecticide resistance, however resistant tobacco budworms did not revert to susceptible until 12 generations under laboratory culture.

TABLE 2. - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
2.2 Determine and compare optimal insecticide application techniques utilizing best available technology (aerial, ground-rig, chemigation, etc.) to improve application methods for improved efficacy and lower environmental impact of currently used chemical insecticides and biorationals.	Conduct experiments using available of new application systems in the field on various crops or in the lab that emphasizes effects of spray volumes, carriers, concentration, droplet size, percent coverage, placement, release rate, etc. on effects of candidate insecticides/biorationals.	X		Field studies of aerial, ground, and chemigation systems were conducted to optimize application technologies. Aerial spray despositions were optimized with higher spray rates and larger droplet sizes and by making applications when temperature and wind speed were low and relative humidity was high. Increasing air speed of turbine aircraft significantly decreased spray drift. Ground sprayer tests indicated that high-volume air-assisted sprayers provided the greatest spray coverage on the underside of cotton leaves as well as deposition on leaves and squares located within the canopy. Pesticide formulation, spray pressure,airspeed, nozzle type and orientation were shown to effect droplet size. A method was developed for demonstrating techniques for reducing spray drift. Non-EC petroleum oil added to several chemigated pesticides increased bollworm mortality and residual activity in cotton, however, addition of oil to *B. thuringiensis* resulted in poorer performance. Application of cypermethrin plus chlorpyrifos via chemigation using rotating sprinklers on drop tubes provided better control of larvae in fresh market corn than conventional application or chemigation with impact sprinklers. Oil carriers were shown to transfer greater amounts of pesticide from cotton leaves to tobacco budworm than water alone.

RESEARCH SUMMARY: ACTION AREA II—CHEMICAL CONTROL AND APPLICATION TECHNOLOGY

Compiled by I. W. Kirk and D. A. Wolfenbarger

LEAD ARRAY: 2.1. Numerous commercially available chemical and biorational insecticides were evaluated in laboratory and field experiments for control of *Heliothis/Helicoverpa* eggs and larvae in cotton, corn, and tobacco. Most of the compounds are highly efficacious at labeled rates. Some of the synthetic pyrethroids gave >80% control at less than half of lowest labeled rates. Combinations of some insecticides at less than recommended label rates provided better control than recommended rates of either chemical alone. Feeding stimulants did not increase mortality with biorationals.

SAFEGUARD ARRAY: 2.1.1. An experimental IGR, RH-5992, was identified as an effective new compound against bollworm and fall armyworm. A new entomogenous nematode, *Steinernema riobravis*, controlled bollworm larvae on corn with varying success.

New varieties of *Bacillus thuringiensis* were effective against larvae of *Heliothis virescens* and *Helicoverpa zea*. Addition of oil to *B.t.* chemigation formulations did not improve efficacy.
Several new antiinsectan natural products have been isolated from sclerotia of *Aspergillus* spp. that have oral activity against *Helicoverpa zea* comparable to that of the commercial synthetic insecticides malathion (an organodithiophosphate) and permethrin (a pyrethroid)).

The nuclear polyhedrosis virus, Af*M*NPV, is infectious to *H. zea* and *H. virescens*. In 1991 small field tests showed *H. zea* and *H. virescens* were approximately equal in susceptibility to residues of Af*M*NPV and the *Heliothis* virus on cotton leaves. By day 7 less than 50% of the original activity was left on leaves when 4 x 1013 PIB/acre were applied. These studies show that Af*M*NPV has potential as a microbial control agent for control of both species. In 1992 small field trials were conducted in California, Arizona, and Mississippi to further elucidate the potential of Af*M*NPV. Field infectivity of Af*M*NPV was demonstrated at the three locations. A fluorescent brightener (M2R) increased field persistence to Af*M*NPV; time to 50% loss of original was extended from 5.5 to 11.5 days at the high Af*M*NPV rate. The addition of a low rate of M2R had no influence on field performance of Af*M*NPV in tests at Stoneville. In laboratory studies M2R increased Af*M*NPV activity by 4.3, and 2.9 fold when fed to *H. virescens* and *H. zea* respectively.

OPTIMIZING ARRAY: 2.1.2a. Several polymeric encapsulations of sulprofos and profenofos were developed to evaluate efficacy against tobacco budworm (TBW), *Heliothis virescens* eggs and larvae in cotton. The polymeric encapsulations varied in efficacy but none of them gave higher egg and larval mortalities than conventional EC formulations in spray-table and greenhouse studies.

Addition of non-EC oil to chemigated formulations of synthetic pyrethroids and organophosphates gave improved bollworm control compared to no-oil chemigation formulations and conventional ground sprayer applications. However, with formulations of *Bt*'s, bollworm control was higher with no-oil chemigation than with addition of non-EC oils. Addition of petroleum diluents to spray mixtures of a synthetic pyrethroid did not increase mortality of susceptible or resistant strains of tobacco budworm larvae in spray table studies.

OPTIMIZING ARRAY: 2.1.2b. Pesticide formulation, spray mixture concentration, surfactants, polymers, spray pressure, airspeed, nozzle type, and nozzle orientation were all shown to affect droplet size of aerially applied pesticides. Relationships between these factors provide applicators with guidance for droplet size control which is important in spray deposition and insecticide efficacy. A simple method was developed for demonstrating techniques for reducing spray drift. The methods were used in spray drift research studies and in a beltwide spray drift reduction demonstration that provided guidance to aerial applicators on selection and operation of aerial spray equipment for optimizing aerial application practices.

Bifenthrin and paraffinic oil mixtures applied to cotton leaves in different droplet sizes showed tobacco budworm larval mortalities occur more quickly with 337 μm droplets compared to 96 μm droplets. Field-scale

studies had higher populations of bollworm and budworm in 30" than 40" cotton. Overall mean spray deposits were higher in 40" cotton. Chlorpyrifos in immiscible oil applied in high volumes of water in sprinkler irrigation systems gave 80-90 percent control of Lepidopterous larvae at half of the lowest recommended active ingredient rate for conventional sprays. Large (74 μm DV0.5) chlorpyrifos/immiscible oil droplets in laboratory-simulated sprinkler irrigations gave more effective larval control than small droplets (6 μm DV0.5). Immiscible oil volume did not affect larval control.

SUPPLEMENTARY ARRAY: 2.1.3a. Experimental methodology was developed to determine the fate of synthetic pyrethroids applied to corn following heavy rainfall. Preliminary studies have shown little chemical residue in plant washoff and field runoff.

SUPPLEMENTARY ARRAY: 2.1.3b. Resistance to four classes of insecticides was monitored with the adult vial test and the spray-table assay on field-collected strains of *H. virescens*. Resistance was variable but often at high levels before, during, and at the end of the cotton growing season. Resistance of tobacco budworm to endosulfan was documented as not synergizable. The presence of metabolic resistance was detected using inhibitors of mixed function oxidases and hydrolytic esterases. Resistance in a laboratory strain of tobacco budworm to cypermethrin and thiodicarb did not revert to susceptible until 12 generations in culture. Relationships between adult vial test mortalities and percentage control of tobacco budworm in field studies were not significant.

SUPPLEMENTARY ARRAY: 2.1.3c. Timing of cypermenthrin sprays to control hatching larvae of *Heliothis/H*elicoverpa in 30 individual fields over two years resulted in greater than 90% control.

LEAD ARRAY: 2.2. Field studies of aerial, ground, and chemigation systems have been conducted to optimize application technologies. Aerial and ground studies with biorationals have shown no major differences between aerial and ground systems in full-season *Heliothis virescens* control.

SAFEGUARD ARRAY: 2.2.1. See progress noted under Safeguard Array 2.1.1

OPTIMIZING ARRAY: 2.2.2. Droplet size, droplet density, spray rate, and active ingredient rate are important parameters in determining tobacco budworm egg and larval mortality. Laboratory and field studies have shown that egg and larval mortalities can be increased or optimized by adjusting these variables for each insecticide. Spray deposition from aerial application of insecticides can be increased with higher spray rates and larger droplet sizes and by making applications when temperature and wind speed are low and relative humidity is high. Laboratory and field studies have shown that efficacy of synthetic pyrethroids can be maintained while increasing droplet size to reduce drift. Ground sprayer tests indicated that several high-volume air-assisted sprayers provided the greatest spray coverage on the underside of cotton leaves. Increased spray rate and level of air assistance increased canopy penetration and spray deposition. Sprays assisted with air (velocities up to 16 m/s) increased fluorescent tracer deposits on the canopy middle up to 92% of that from the canopy top, and resulted in significant increase in bifenthrin on leaves and squares located within the canopy.

Sprays of bifenthrin and parafinic oil mixtures were applied on cotton leaves to investigate droplet size effects on the mortality of tobacco budworm; droplet size did not influence mortality 117 hours after treatment, but mortality was significantly affected by bifenthrin rate.

Drift from a turbine aircraft traveling at 218 km/h was significantly greater than at 241 and 265 km/h. Drift from an aerial spray boom equipped with Chimavir winglets was significantly less at a release height of 3.0 m as compared to release heights of 4.6 m and 6.1 m. Deposition of bifenthrin on cotton was increased by the use of Chimavir winglets mounted to the aerial spray boom.

SUPPLEMENTARY ARRAY: 2.2.3. The transfer of bifenthrin/carrier combinations from cotton leaves and inert surfaces (glass and wax) showed that oil carriers transferred significantly greater (up to 50%) amounts of bifenthrin from cotton leaves to tobacco budworm (TBW) than water. The transfer of bifenthrin using vegetable and hydrocarbon based oils from cotton leaves to larvae was affected by carrier viscosity. Feeding tests showed that pyrethroid-resistant TBW were more tolerant to gossypol than susceptible TBW. Differences in performance

of TBW in feeding bioassays were attributed to differences in the amount of time since outcrossing to the wild.

Progress was made on all arrays listed in Action area II and research appears to be on track as identified in the National Suppression Plan.

BREAKOUT SESSION SUMMARY

This group was encouraged by the tone of the general discussions and presentations, and recognition of the need to include application technology in the early phases of research plans and programs for all control strategies. Recognition of the need to explore promising control strategies on an area-wide basis was also noted favorably by those attending the break-out session for Action Area 2. There are several control strategies, including biologicals and some chemicals, that have been researched by ARS over the years that clearly can not be evaluated except on an area-wide basis. ARS is a partner in an agricultural research discovery, delivery, and feedback system with APHIS, ES, and CSRS cooperators. ARS must take a leadership role in implementing an area-wide control strategy on a pilot basis. The *Heliothis/ Helicoverpa* complex would be an appropriate pest on which to implement this approach. The task will be difficult because of the wide host range, mobility, and plastic genetics of these pests. Understanding the vulnerabilities in *Heliothis/Helicoverpa* biology and migration, and low-level sampling methodology will be critical to success of an area-wide pilot program. Hopefully, those that have worked on the most promising biocontrol strategies can provide the basis for an area-wide pilot control program. Researchers in Action Area 2 expect that chemical control will be a part of an area-wide initiative. They also expect that application technology will be a vital part of appropriate delivery of all types of control agents in an area-wide pest management program. These researchers also believe overall coordination and management of action area inputs to a given area-wide pest management strategy will be critical to success of such an effort. These researchers are interested in contributing and participating to make such a program successful.

Relative to specific research needs, participants agreed that additional research should be conducted on biologicals and IGRÆs with a focus on developing area-wide management programs for either or both species. It was also agreed that additional research effort is needed in the field of spray deposition and drift. The concerted efforts of the pesticide chemical companies through the Drift Control Task Force illustrates the concern of our lack of knowledge in this field relative to current environmental/ecological focus. Research directed by the Task Force has shown that current methodologies for measuring drift are inadequate for proper characterization of the phenomenon.

It was generally felt that existing technology utilizing both adult and larval bioassays were adequate for resistance monitoring, even though there is some disagreement as to our ability to measure resistance in adult populations. Further, concern was expressed as to what constitutes field control of tobacco budworm. Since it is assumed that resistance normally develops on a field-by-field basis and the bollworm has not exhibited pesticide resistance, additional research should focused on developing immunology techniques for rapid and inexpensive identification of the species composition within fields to aid in making decisions concerning the chemicals to be applied for control. Participants further agreed that additional research was needed to assess the impact of migration on the dispersal of resistant populations.

Action Area III. ECOLOGY AND POPULATION DYNAMICS

Coordinators: J. D. Lopez and T. Popham

INVESTIGATOR'S NAME(S): P. D. Lingren[a], J. R. Raulston[b], T. W. Popham[c], W. W. Wolf[a] and P. S. Lingren[d]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b] USDA, ARS, CIRU, Weslaco, TX; [c] USDA, ARS, Biometrics, Stillwater, OK; [d]Current address: Dept. of Entomology, Louisiana State University, Baton Rouge, LA

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.1 Quantify preflight activities

OPTIM ARRAY: 3.1.1 Optimize technology for observing preflight activities

DATES COVERED BY REPORT: September 1991 - July 1993

PROGRESS REPORT: Various types of low light level video cameras, optical, and nocturnal viewing systems have been investigated for use in making unobtrusive observation of preflight activities of newly emerged moths. Several systems have been identified for possible use but to date none have been procured other than video systems and night vision goggles which work very well for making such observations of newly emerged moths. Following emergence, the moths move to corn stalks, grass or other debris, crawl upon it and place themselves in a horizontal position. There they expand and dry their wings and feed on exudates from the corn plant. As the time after emergence progresses, the moths tend to move further up the plant. Wing vibrations can begin one to three hours after emergence prior to short flights of <5 m up plants or to another plant. Very few moths fly distances >5 m. Many go down into the ground litter at about sunup and remain there until the following evening until about one half hour after sundown. A large majority of these moths leave senescing corn or other night resting sites before midnight the night after emergence. Thereafter, they either fly about canopy level or make spiral-like flights while flying out of an observation range of 100 m altitude. A few moths fly 50-150 m to heavy foliage at 2-4 hr after emergence.

FY94 & FY95 WORK PLANS: Fed and nonfed moth flight activity will be determined following emergence utilizing night vision equipment of various types (tracking radar and optical-mechanical observation techniques developed by the unit).

INVESTIGATOR'S NAME(S): J. K. Westbrook

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.1 Quantify pre-flight activities

OPTIM ARRAY: 3.1.2b Determine meteorological influences on newly-emerged adults

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Detailed microscale weather data have been collected at the sites of adult emergence. However, this data has not yet been analyzed to determine the meteorological influences on pre-flight behavior.

FY94 & FY95 WORK PLANS: Automated weather stations instrumented with several anemometers and wind vanes at various heights from near ground level to 10m will be deployed in 40m x 40m plots of sweet corn and other field crops. The weather stations will be located at three locations within the plot to establish lateral gradients of the atmospheric wind flow and dispersion. Instrumented pilot balloons or a tethered balloon system will be deployed to measure the vertical profile of wind velocity, air temperature and relative humidity from the surface to heights of at least 100m above ground level where flying insects can be observed. Night vision observations of newly emerged adults will be analyzed to determine the behavior of the insects relative to feeding, mating, ovipositing and migrating.

INVESTIGATOR'S NAME(S): K. R. Beerwinkle[a], T. N. Shaver[a], P. D. Lingren[a], J. D. Lopez, Jr.[a], and J. R. Raulston[b]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b] USDA, ARS, CIRU, Weslaco, TX

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.2 Determine adult response to plants and plant volatiles

OPTIM ARRAY: 3.2.2 Determine and define interaction of adults with local plant populations

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Free choice olfactometer systems (2 and 6-choice) were developed for bioassaying the feeding attractiveness of various plant volatiles to Heliothis/Helicoverpa spp. adults in the laboratory. Methods and techniques have been developed for bioassaying volatiles from various sources including bouquets of plant flowers and plant parts; whole plants; chemical extracts from plant parts; chemical compounds derived from vacuum collected plant volatiles; and various synthetic chemical mixtures. Some of the more important results obtained from laboratory bioassay tests with the olfactometers include: (a) Volatiles from ergot-infected dallisgrass seed heads and from the flowers of three *Guara* spp. (*G. drummondi*, *G. suffulta*, and *G. longiflora*), citrus, oak, willow, and several other blooming plants have shown activity as feeding attractants for both field-collected and laboratory-reared *H. zea* adults. (b) Dose/response relationships for feeding attractiveness of *G. drummondi* blooms, *G. suffulta* blooms, *G. longiflora* blooms, and ergot-infected dallisgrass seed heads have been established for *H. zea* adults in the 2 and 6-choice olfactometer systems. (c) Some synthetic chemical compounds which were mimics of compounds identified in attractive plants have shown activity as attractants. In a field study, feeding *H. zea* adults were observed to be highly attracted to the honeydew exudates of ergot on infected florets of dallisgrass (Beerwinkle et al. 1993). The study was conducted in an area adjacent to a corn field where *H. zea* were emerging. Moths began feeding on the ergot at dusk. Feeding densities increased rapidly to peak at $<$ 1h after sunset and then declined to relatively low levels by 2 h after sunset. Dissection analyses of sampled females showed that 95% were unmated, indicating that the characteristic age of the feeding moths was $\leq$ 1d.

FY94 & FY95 WORK PLANS: Research will continue to evaluate and quantify the attractiveness of volatiles from candidate plant materials and synthetic chemical mixtures to identify attractive chemicals and optimize their formulation. Test equipment and procedures will be further developed, modified, and adapted as necessary to achieve efficient bioassays of possible attractants.

INVESTIGATOR'S NAME(S): D. E. Hendricks

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 3. Ecology & Population Dynamics

LEAD ARRAY: 3.2 Determine adult response to plants and plant volatiles

OPTIM ARRAY: 3.2.2 Determine and define interaction of adults with local plant populations

DATES COVERED BY REPORT: May 1991-December 1992

PROGRESS REPORT: Velvetleaf, *Abutilon theophrastii* Medikus, and cotton were inspected for eggs and larvae of bollworms and tobacco budworms in both 1991 and 1992 from April through November. Analyses of data from these surveys indicated that velvetleaf sustained the progeny of bollworm and tobacco budworm moths that had emerged from local overwintered pupae, or from f1 generations developed on other springtime wild host, or had immigrated into the area in the early springtime. Velvetleaf also supported major peaks of eggs of both species during the cotton-growing season and substantial tobacco budworm larval populations from late September to November of both 1991 and 1992. Eighty percent (80%) of the eggs found on velvetleaf during the sampling period of both years were found at the top of young milk-stage fruit (seed pods); this indicated that a tactile or/and chemical oviposition stimulant may be present at the top of young velvetleaf fruit. The remaining 20% of the eggs were found on terminal or young foliage and on bracts of flowers. In 1991, blooms and buds of this weed provided attractive feeding and oviposition sites for females of both species from about 30 days before to 40 days after peak blooming of cotton. Greater numbers of bollworm eggs were found on velvetleaf in June of 1992 than during the same period in 1991. The parent moths of these eggs apparently had emerged from fields of matured corn. The March-to-May corn-growing season of 1991 was eliminated by flooded soil conditions. During the cotton-growing seasons of both years, velvetleaf plants were host to about 20 times the number of tobacco budworm eggs and 30 times the number of bollworm eggs found on cotton. Sex ratios determined from eggs and larvae collected from velvetleaf and cotton were about 1:1 for both species. Velvetleaf growing in or near cotton fields is considered the single most important weed host that sustains late-season tobacco budworm larval populations that ultimately overwinter as pupae in the mid-delta region of the Mississippi river. Destruction of this weed each fall, after cotton harvest, is recommended as an annual agronomic practice that could reduce significant numbers of tobacco budworms destine to emerge the following spring.

FY94 & FY95 WORK PLANS: Continue survey and inspection of wild host plants that support tobacco budworm or bollworms noting which plant structures are used most commonly for oviposition.

INVESTIGATOR'S NAME(S): J. D. Lopez, Jr., P. D. Lingren, and T. N. Shaver

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.2 Determine adult response to plants and plant volatiles

OPTIM ARRAY: 3.2.2 Determine and define interaction of adults with local populations

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Four malaise traps (6m) with their long axis oriented east-west so as to intercept the predominant wind direction were installed in a large fallow field and baited with different numbers of potted flowering greenhouse-grown *Gaura suffulta* plants (0, 2, 10, and 24). To replicate the test over time, the treatments were moved one position each night after the initial treatments were randomly assigned. Although some noctuids were captured especially soybean loopers which were apparently coming from a nearby cotton field, insufficient numbers of moths were captured to determine the effect of patch size on adult noctuid attraction. A trend was observed for higher captures with the greater number of plants. Apparently, the test will have to be conducted in areas in which there is considerable adult activity such as a maturing corn field when corn earworm adults are emerging. Captures of corn earworm and tobacco budworm in malaise traps placed in areas with different concentrations of ergot-infected dallisgrass were directly related to the concentration of the ergot-infected dallisgrass. The largest numbers were captured in a ditch with a dense stand of the ergot-infected dallisgrass. Captures in 1m malaise traps placed along roadside stands of flowering *G. suffulta* in the spring 1993 captured very few corn earworms which probably reflects low corn earworm activity in those areas, however, the *G. suffulta* stands were meager due to a late freeze and very wet conditions which caused excessive growth of winter grasses. Field-collected males and females and pheromone-captured males showed a strong positive proboscis extension response to *G. suffulta* nectar collected from greenhouse plants. Refractometer readings of the nectar indicated about 30% dissolved solids. Large numbers of adult corn earworms were captured at night by net while feeding on blooming *G. suffulta* in a field plot, but the number decreased apparently as the corn in the area became attractive. While collecting adults from a silking corn field that was adjacent to a grassy slough, considerable corn earworm feeding activity was observed on the seedheads of a coarse rhizomatous grass growing in the slough. Inspection of the seedheads indicated the presence of a sticky material. It is possible that the seedheads were infected with an ergot-type disease which resulted in the production of honeydew. Specimens of the grass have been collected and prepared for identification.

FY94 and FY95 WORK PLANS: Malaise traps will continue to be used to evaluate the response of corn earworm and tobacco budworms to different concentrations of plants that have been shown to be attractive for feeding to determine factors that influence response. Emphasis will be on conducting the evaluations in areas with high adult activity. The feeding response of both laboratory-reared and field collected corn earworm adults to nectar from various species of *Gaura* will be evaluated relative to concentration. The nectar composition will be determined to identify key components contributing to the high feeding stimulatory activity. The coarse slough grass will be identified and nocturnal observations made to determine the pattern of activity and the associated feeding behavior. If a strong feeding stimulant is observed, efforts will be made to identify the composition of the stimulant. The response of corn earworm to ergot honeydew will also be evaluated relative to factors influencing feeding response.

INVESTIGATOR'S NAME(S): S. D. Pair

AFFILIATION & LOCATION: USDA, ARS, SCARL, Lane, OK 74555

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.2 Determine adult response to plants and plant volatiles

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Potential adult food plants were identified from analyses of pollen loads.

FY94 & FY95 WORK PLANS: Investigate attractancy of identified plants.

INVESTIGATOR'S NAME(S): J. K. Westbrook

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.2 Determine adult response to plants and plant volatiles

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: An automated weather station has been deployed in the center of a 20m x 20m plot of sweet corn. The weather station is instrumented with anemometers and wind vanes at several heights from 0.43m to 3.20m above ground level to measure the vertical profile of wind velocity within the developing corn canopy. Further, a three-dimensional propeller anemometer and three hot film anemometers are included among the sensors to measure high-frequency turbulence. The weather station in the center of the field and one at 2m outside the prevailing upwind perimeter of the plot are instrumented with air temperature, soil temperature, relative humidity and soil moisture sensors. Atmospheric dispersion values will be calculated to determine the amount of lateral and vertical mixing that could disperse plant derived allelochemicals above and under the corn canopy.

FY94 & FY95 WORK PLANS: Automated weather stations instrumented with several anemometers and wind vanes at various heights from near ground level to 10 m will be deployed in 40m x 40m plots of sweet corn and other field crops. The weather stations will be located at three locations within the plot to establish lateral gradients of the atmospheric wind flow and dispersion. An electrostatic air filter will be modified to ionize a known rate of air molecules. Five bipolar ion collectors will be constructed and attached to vacuum pumps. The ion collectors will be deployed in an orthogonal x-pattern (20m x 20m) with one axis parallel to the rows of corn, and will detect atmospheric ions as tracers of dispersing plant derived allelochemicals. Quantification and modeling of the concentration of dispersing ions will establish the high-frequency spatial distribution of neutrally-buoyant substances, and can be modified to account for differences in density and other relevant physical properties of various plant derived allelochemicals. Mean dispersal rates of various plant derived volatiles will be measured by vacuum sampling of air through an adsorbing substrate for several hours at five locations in the field plots and analyzing the concentration of the allelochemicals by gas chromatography / mass spectrometry. Vaporization rates of various allelochemicals from several substrates used as lures will be measured in environmental chambers under controlled temperature, relative humidity and wind speed conditions. Subsequently, we will observe insect behavioral response to dispersing allelochemicals in the field plots using night vision equipment.

INVESTIGATOR'S NAME(S): K. R. Beerwinkle[a], J. D. Lopez, Jr.[a], P. D. Lingren[a], J. A. Witz[b], P. G. Schleider[a] and R. S. Eyster[a]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b]Formerly USDA, ARS, PMRU, College Station, currently, Texas Transportation Institute, Texas A&M University, College Station, TX

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.3 Determine migratory and trivial flight initiation and termination

OPTIM ARRAY: 3.3.2 Develop equipment and technology for qualitative and quantitative behavioral observation of boundary layer flight

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Nocturnal aerial insect flight activities at altitudes between 30 and 900 m were monitored with x-band scanning radar during the spring, summer and fall seasons of 1988 and 1989 in the Brazos River Valley of Burleson County near College Station, TX (Beerwinkle et al. 1991). Surface meteorological parameters were measured continuously with weather station instrumentation, and radiosondes carried aloft by weather balloons were used to measure upper-air temperatures and wind conditions. Aerial volume density patterns and flight behaviors observed with radar varied from night to night because of the many biological and meteorological variables involved, but certain seasonal trends and characteristics of insect movement behavior became apparent during the course of the research. Nightly local dispersal flights at dusk were the norm, especially during the summer. Large numbers of insects were typically airborne for 1 to 2 h beginning about one-half hour after sunset with some of them reaching altitudes of 800 m or more where wind speeds were typically greater than 30 km/h. Several apparent long-range migration-type insect movement events were observed in which insects were concentrated in layers in high-speed, low-level wind jets that were apparently associated with nocturnal upper-air temperature inversions. Migration-type movement of insects tended to be south to north in the spring and early summer and north to south in the fall.

An automated, vertical-looking radar system was operated continuously 24 h per day in the Brazos River Valley of Burleson County near College Station, TX, during most of 1990 and 1991. Airborne targets that were detected with the radar in 64 range intervals, covering the range from 100 to 2400 m, were automatically counted, and the accounts accumulated over successive 5-min time periods and stored by the computer. Considerable data were collected and some of it is still being analyzed (manuscript in preparation). A brief summary of the results follow: (1) Aerial insect densities were consistently the highest at the lower altitudes from 100 to 500 m, and they decreased with increasing altitude, especially above 600 m. (2) Daily and seasonal periodicities in airborne insect concentrations were apparent both at the lower altitudes (100-500 m) and the upper altitudes (500-2400 m). (3) some upper-air (altitudes > 500 m) insect flight activity detected during the day, but there was considerably more upper-air flight activity was detected at night when noctuids would be flying.

FY94 & FY95 WORK PLANS: K. R. Beerwinkle has no firm plans formulated for research in these areas.

INVESTIGATOR'S NAME(S): J. D. Lopez, Jr., K. R. Beerwinkle, and P. D. Lingren

AFFILIATION AND LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.3 Determine migratory and trivial flight initiation and termination

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: A computer-controlled flight mill system with 32 units was developed. The units are operated in a humidity- and temperature-controlled room with axillary cooling capability in which temperatures from $<10°C$ to $>40°C$ can be obtained. Sunset and sunrise are simulated with a computer-controlled lighting system. Temperature, humidity, barometric pressure and light level are independently logged in the flight mill room during flight evaluation periods. The effect of source of the moths (laboratory-reared or field moths captured in pheromone traps), sex, age, mating status, temperature, humidity and light level were evaluated for their effect on *H. zea* flight. Laboratory-reared adults were more uniform in their flight potential than field moths which tended to show a bimodal distribution for flight potential with a proportion of the moths being able to fly continuously for considerably long periods. No major differences due to age, sex, or mating status of laboratory-reared females were found. Younger laboratory-reared females (1 and 2 day-old) flew greater distances than the 3 and 4 day-old at temperatures of 10 and 15°C; however, all ages flew similar distances at 20 and 25°C. Temperatures above 25°C and up to 40°C were not conducive to long-duration flight and mortality was extremely high especially for the 4 day-olds. Low humidity especially in conjunction with high temperatures resulted in very high mortality levels during flight evaluations. Light level was inversely proportional to flight initiation during simulated sunset; however, field moths were better synchronized with light level for flight initiation than the laboratory-reared moths especially when the light transition period was extended. Allatectomy of newly emerged *H. zea* females which prevented egg maturation did not result in increased flight when compared to sham-operated females indicating that a migration phase was not induced solely by the absence of juvenile hormone. Rearing *H. zea* females under simulated spring and fall temperature and photoperiodic conditions did not increase flight potential compared to standard rearing conditions (27°C, 14: 10h L:D constant).

FY94 & FY95 WORK PLANS: Improvements to the flight mills will be made so that the adults will have the opportunity to stop flying by providing a landing platform when flight decreases below a certain speed. Heretofore, no resting platform was provided to allow the moths to stop flying in a relatively natural manner. Emphasis will be given to the flight evaluation of field moths (pheromone-trap captured, collected directly from the field, or from emergence cages) especially as these are affected by host plant and host plant volatiles. Other improvements will be made to the flight mill system to determine its potential for bioassaying feeding attractants/stimulants as well as the effect of such factors as wind speed on flight.

INVESTIGATOR'S NAME(S): P. D. Lingren[a], J. R. Raulston[b], T. W. Popham[c], W. W. Wolf[a], P. S. Lingren[d] and J. K. Westbrook[a]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b] USDA, ARS, CIRU, Weslaco, TX; [c]USDA, ARS, Biometrics, Stillwater, OK; [d]Current address: Dept. of Entomology, Louisiana State University, Baton Rouge, LA

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.3 Determine migratory and trivial flight initiation and termination

OPTIM ARRAY: 3.3.2 Develop equipment and technology for qualitative and quantitative behavioral observation of boundary layer flight

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Techniques were developed to observe and describe the flight behavior of adult corn earworms up to 100 m above ground level (AGL) for unlimited horizontal distances. Observation and tests showed significant differences in flight of moths due to age after emergence and following mating. During take-off, moths exhibited a spiral-like behavior to a mean altitude of 50 m. At that point they oriented (apparently to the wind) and flew in a more or less straight line with a less steep ascent angle ($\bar{x}$ = 43.4 vs. 23.4°). Likewise, their ascent rate was faster during spiral-like flight than after orientation to the wind ($\bar{x}$ = 6.8 vs. 2.5 m/s). Mean moth velocity during flight was 3.5 m/s. Newly emerged moths seldom exhibit spiral-like flight and fly short distances within and near the plant canopy.

FY94 & FY95 WORK PLANS: Two radars capable of tracking individual moths up to 1.6 km have been obtained and will be utilized to study individual moth orientation and navigation. Flight mills will be utilized to study moth flight over water and various other terrain. Results will be integrated with meteorological information for predicting origin and fall-out of migrating moths. LORAN and radar observation of instrumented tetroons placed in a migrating cloud of moths has shown moth fall-out in clear air subsidence as well as in the presence of heavy thunderstorms. This information will be used in conjunction with the development of area-wide management strategies for the corn earworm and other insect species and in development of the science of aerobiology.

INVESTIGATOR'S NAME(S): J. K. Westbrook

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.3 Determine migratory and trivial flight initiation and termination

SUPPL ARRAY: 3.3.3 Identify meteorological events that affect flight initiation and orientation

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Several $1m^3$ and $2m^3$ tetrahedral-shaped, mylar balloons (tetroons) were launched from Weslaco (June 1992-1993), Eagle Lake (July 1992) and Plainview (August 1992), TX during emergence of adult corn earworm from commercial corn fields. The tetroons were ballasted to drift at altitudes of insect flight (approximately 700m above ground level), and launched nightly at the time of flight initiation (about 0.5h after sunset). Each instrumented tetroon was tracked for up to 9h by either the ARGOS satellite or a radio-equipped van. Four of the six tetroons tracked for one night from Weslaco in 1992 were located at a mean displacement of 299km $\pm$ 95km and direction of 339° $\pm$ 7°. A three-successive-night (27h) tetroon trajectory originated at Eagle Lake and passed near Atlanta, TX, Jonesboro, AR and Nashville, TN, on successive nights for a total displacement of 1394km. Several $1m^3$ tetrahedral-shaped, mylar balloons (tetroons) were launched from Plainview, TX during emergence of adult corn earworm from commercial corn fields in August 1992. The tetroons were ballasted to drift at altitudes of insect flight (approximately 700m above ground level), and launched nightly at the time of flight initiation (about 0.5h after sunset). Seven tetroons were tracked from Plainview all night or until atmospheric subsidence or precipitation downed the tetroons. An airborne entomological radar and two mobile ground-based entomological radars monitored insect flight in the vicinity of the drifting tetroon. On several nights, at least one entomological radar was operating in the vicinity of the tetroon as it was downed by atmospheric subsidence or precipitation.

FY94 & FY95 WORK PLANS: Field activities will consist of two primary periods. The first period will coincide with the citrus blooming period (mid-March through mid-April) in the lower Rio Grande Valley (LRGV). Citrus pollen provides a natural mark to moths that feed on it. The second period coincides with peak moth emergence from whorl- and fruiting-stage corn in the LRGV of Mexico and Texas. Food dyes or other types of markers will be investigated for internal marking of newly emerged adults. Emerging adults will be self-labeled by feeding on citrus in the LRGV and by markers previously applied to the soil or corn stalks in a 100 ha area of corn in the LRGV. Adults will be captured in the LRGV and anticipated fall-out areas to determine the number of marked moths that have exited and entered these areas, respectively. A radio-equipped van and the ARGOS satellite will track instrumented tetroons to provide an atmospheric trajectory data base which represents the displacement of centers-of-mass of diffusing moth "clouds" for long distances. The tetroon altitude will be based on the initial flight altitude of the moth "cloud" measured by entomological radars. Tracking of the tetroon will measure the atmospheric pressure, temperature and humidity of the ambient air, and the three-dimensional dispersal of an air parcel representing the center-of-mass of a passive moth "cloud." Tracking will continue until dawn. Insect behavior that affects migration distance and dispersal will be measured with ground-based and airborne radars. Behavior such as take-off and ascent at the source, insect flight speed, insect orientation, vertical distribution (insect layering), and mean flight altitude are derived from radar data. The spatio-temporal dispersion of insects from corn will be measured with radar and aerial sampling during the period of emergence and dispersal from the mature crop. Personnel tracking the tetroons will notify radar operators and entomologists to coordinate the downwind deployment of monitoring crews during the nightly migration events to measure fall-out. Aerial sampling will identify species as well as correction factors for application to radar data if other species occur in significant quantity. Airborne radar measurements of horizontal insect distributions will be compared with insect dispersal estimated by tetroons to differentiate between atmospheric dispersal and active flight.

INVESTIGATOR'S NAME(S): W. W. Wolf

AFFILIATION & LOCATION: USDA ARS CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and population dynamics

LEAD ARRAY: 3.3 Determine migratory and trivial flight

OPTIM ARRAY: 3.3.2 Develop equipment and technology for qualitative and quantitative behavioral observation of boundary layer flight

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: At most radar locations, insect flight activity rapidly increased during the crepuscular onset of flight (30 min after sunset), reached a peak (1-1.5 hr after sunset) and then decreased to 10-50% of maximum during the night. One interpretation of the late night "back-ground" activity is trivial flight of nonmigratory individuals.

An optical disk drive was acquired, and software developed for real- time digitization of airborne radar data. This technology improves the quality of the data and saves time of digitizing from analog magnetic tape. The airborne radar is an important tool for quantifying flight in the boundary layer. A tracking radar was acquired and can automatically track an individual corn earworm moth to 1.3 km. The radar provides three dimensional coordinates of moth position. These coordinates can be used to calculate the moth's displacement vector. If the wind vector is available for the same time and altitude, then moth heading and air speed can be calculated. Moth heading, air speed and flight altitude are behaviors that affect the displacement and direction of dispersal.

FY94 & FY95 WORK PLANS: The tracking radar will be mounted on a trailer and located downwind of corn earworm source areas. Targets will BE tracked, wind vectors measured, and insect headings, air speed, and altitude calculated. Individual corn earworms will be released and tracked to determine behavior during ascent, and changes in flight behavior with age of the moth. These experiments will be designed to detect trivial vs. migratory flight. Moths that have been exercised on flight mills for one or more nights will be released and tracked to simulate multiple-night flight behavior.

INVESTIGATOR'S NAME(S): A. C. Bartlett

AFFILIATION & LOCATION: USDA, ARS, WCRL, Phoenix, AZ

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.4 Determine origins of adult populations

OPTIM ARRAY: 3.4.2d Analyze genetic variation in natural populations

DATES COVERED BY REPORT: 1992-1993

PROGRESS REPORT: No research conducted in this area during this period.

FY94 & FY95 WORK PLANS: If specimens are acquired from co-operators as requested, I will conduct RAPD-PCR analysis of *H. virescens* for the development of fingerprints in preparation for the identification of origin of individual moths.

INVESTIGATOR'S NAME(S): D. E. Hendricks

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS
ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.4 Determine origins of adult populations

OPTIM ARRAY: 3.4.2a Identify and characterize source population zones

DATES COVERED BY REPORT: February 1991-December 1992

PROGRESS REPORT: Procedures for collecting bollworm and tobacco budworm moths from survey traps baited with pheromone installed throughout the U. S. were established early in 1991. A method of shipping these living moths, via Federal Express, was developed. Moths collected by 28 cooperative entomologists during a 2-year period were shipped monthly throughout the local growing seasons to S. Karl Narang, USDA-ARS, Fargo, ND. A method of "finger printing" the moths was developed that characterize the moths collected at each different geographic locations. This method of identification will help identify the geographic origin of moths that have dispersed and are caught in traps, perhaps great distances from where they emerged.

Analysis of genetic material from tobacco budworm moths collected from Texas, Louisiana, Mississippi, Arkansas, and Georgia showed that within local populations, budworm moths typically traveled 8 km or less during a two-week period in the middle of the growing season, and resistance to commonly used insecticides are better dealt with inside the perimeter of local areas of a 8 km diameter. This work supports the inference that single moths dispersing beyond the limits of this area would not significantly contribute to development of resistance to insecticides in other regions. Initial design of this project was conceived by James Mallet and Amy Korman, Miss. St. Univ., and starch gel electrophoretic analyses were used to determine differences or similarities of moths collected.

FY94 & FY95 WORK PLANS: Specimens collected from the mid-Delta Region of the Miss. River will be sent to S. Karl Narang for analysis to develop finger printing techniques and to determine geographic origins of both species.

INVESTIGATOR'S NAME(S): P. D. Lingren[a], V. M. Bryant, Jr.[b], J. R. Raulston[c], J. K. Westbrook[a], J. F. Esquivel[a] and G. D. Jones[a]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b]Palynology Laboratory, Texas A&M University, College Station, TX; [c]CIRU, Weslaco, TX

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.4 Determine origins of adult populations

OPTIM ARRAY: 3.4.2e Determine pollen loads on adults to determine possible origin

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Captured insects are being analyzed for pollen using scanning electron microscopy. Pollen provides a natural marker for determining the host plant feeding range and migratory activities of noctuids. *Citrus* and *Pithecellobium* pollen have been identified on corn earworm and cabbage looper moths captured in Oklahoma. The nearest source for these pollen types is the Lower Rio Grande Valley of Texas, and associated weather systems strongly suggest that corn earworm and cabbage looper moths contaminated with these pollen types had moved at least 700 km northward. Oak and willow were also found to be highly attractive to corn earworm, cabbage looper, and celery looper. Similarly, Citrus has been used as a key indicator of corn earworm migratory activity. Citrus pollen was identified on 3% of pollen contaminated moths collected in Oklahoma during the spring of 1990. A major freeze during December 1989 drastically reduced or eliminated *Citrus* blooming during the spring of 1990 in the Lower Rio Grande Valley, northeastern Mexico, Louisiana, and central Florida. None of the moths collected in the Lower Rio Grande Valley after the freeze were *Citrus* pollen contaminated. This, in conjunction with the effects of the 1989 freeze, suggests that Citrus pollen contaminated corn earworm moths did not originate in the Lower Rio Grande Valley. Evaluation of calculated trajectories (72-h continuous and discontinuous flight), synoptic weather maps, and upper-air transport opportunities suggest southern Florida, the Bahamas, Cuba, Yucatán Peninsula, and northern Central America as potential source areas $\geq$1,515 km from the capture site. If these are indeed source areas, insects would be required to traverse the Gulf of Mexico and fly day and night over water. UV light reflection is similar in the atmosphere and water while UV reflection is very different over land. Wind and UV light reflection appear to be probable parameters used by moths for navigation during flight. Oak, willow, and Asteraceae pollen were also commonly found on contaminated moths during 1990. Bioassays have confirmed that blooming plants from every pollen type found on contaminated moths are highly attractive to corn earworm as an adult food source. Pollen attached to moths is a good indicator of the host plant feeding range of corn earworms.

FY94 & FY95 WORK PLANS: Moths have been collected from various locations across the US and Mexico and the pollen on these moths will be identified to help determine moth source zones. Blooming dates of various host plants of feeding adult corn earworm will be determined in relation to climatic zones from Mexico into the northern US to enhance the determination of specific pollen sources. The number and types of moths feeding on Citrus in the Lower Rio Grande Valley will be determined. A key to the *Citrus* pollen of the Americas will be developed using morphological and biological plant characteristics. Meteorological, radar, and entomological studies will be utilized along with natural pollen markers to determine fall-out zones of migrating moths. Results from these studies will be utilized to determine source populations, impact of migrants in recipient zones, models for predicting migratory routes and development of area-wide management strategies for corn earworm.

INVESTIGATOR'S NAME(S): J. D. Lopez, Jr.[a], S. K. Narang[b] and D. E. Hendricks[c]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b]USDA, ARS, BRL, Fargo, ND; [c]USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA:	3	Ecology and Population Dynamics
LEAD ARRAY:	3.4	Determine origins of adult populations
OPTIM ARRAY:	3.4.2.a	Identify and characterize source population zones
OPTIM ARRAY:	3.4.2.b	Utilize standardized sampling procedures in multiple cropping systems across geographical regions to determine chronology of developing populations
OPTIM ARRAY:	3.4.2.c	Determine regions in Mexico that may influence development of U. S. populations in the Spring
OPTIM ARRAY:	3.4.3.b	Develop efficient marking techniques for tracing moth population origin

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Cooperators from different parts of the U. S. where *Helicoverpa zea* and *Heliothis virescens* occur were identified to provide samples of males captured in pheromone traps for genetic analyses to determine if any differences could be identified that would help characterize field populations relative to origin. The cooperators included a number of ARS personnel such as Larry Chandler, Jimmy Raulston, Sam Pair, and Bill Showers as well as numerous employees from different states. Moth samples were collected periodically during the 1991 and 1992 growing seasons and shipped alive via overnight delivery to Karl Narang for accumulation of frozen samples for analysis. Analyses of these samples is continuing. In late April, 1992, collections of both *H. zea* and *H. virescens* were made in Mexico with the help of Jesus Loera of Rio Bravo, Mexico. Males were collected at Anahuac (close to Laredo, TX), Zaragosa (close to Eagle Pass, TX), Torreon, Delicias, Hermosillo, and Ciudad Obregon and were shipped to Fargo, ND. During the trip, samples were also collected at Anthony and Lordsburg, NM, and Avra Valley and Yuma, AZ. Although *H. zea* males were captured in relatively high numbers in all the areas, the capture of high numbers at Delicias was surprising, Alfalfa which is grown extensively in the region may be a host for the build-up of populations during the winter. Extremely high numbers of *H. virescens* males were captured adjacent to a garbanzo bean field in an agricultural area west of Hermosillo and at the Research Station area close to Ciudad Obregon. Populations of *H. virescens* in the area are extremely high during early season and decrease as the season progresses which is opposite of what is observed in most areas of the U. S. where it occurs. Contacts were made at most of the areas in Mexico from which moths were collected if additional samples from specific areas are needed. Jesus Loera or Rio Bravo, Mexico could coordinate these collections. No research was conducted on the development of efficient marking techniques for tracing moth population origin.

FY94 & FY95 WORK PLANS: Further research is pending the outcome of analyses of the samples collected as part of the cooperative effort in the U.S. and the collection trip to Mexico. Additional samples will be collected from specific areas if a need is identified as a result of the analyses. Otherwise, no additional research along this lines is planned.

INVESTIGATOR'S NAME(S): S. K. Narang[a], M. Degrugillier[a], and J. D. Lopez, Jr.[b]

AFFILIATION & LOCATION: [a]USDA, ARS, BRL, Fargo, ND; [b]USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.4 Determine origins of adult populations

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Mitochondrial DNA RFLP and allozymes studies were undertaken to determine whether geographical populations could be differentiated and genetic markers be identified to determine population origins. Samples of *Helicoverpa zea* from Mexico (Zaragosa, Hermosillo and Delicias) and US (Corvalis, OR; Ankeny, IA; College Station and Weslaco, TX) were analyzed for mtDNA RFLP patterns using 35 restriction enzymes (REs). Fifteen REs produced polymorphic patterns in one or more populations.

The Mexican populations were more variable than U.S. populations. Eight REs (*Alu* I, *Ase* I, *Dra* I, *EcoR* I, *Hinf* I, *Hpa* II, *Sau* 3AI, and *Sca* I) produced more mtDNA RFLP patterns in Mexican than in US populations. However, the reverse was true for four REs, *Hind* III, *Hpa* I, *Rsa* I and *Sau* 96 I. The average number of patterns per RE was 2.73 in Mexican as compared to 2.33 in the US populations.

Considering the three collections from Mexico as one group and four from US as the second group, both intra and inter-group mtDNA RFLP variations in low frequency patterns were observed. However, all populations shared the same most common patterns. In addition, frequencies of *Alu* I-B, *Hpa* II-B & *Mbo* I-B in Zaragosa (0.18, 0.06 & 0.0 respectively) were significantly different from those of Delicias (0.52, 0.22 & 0.14) and Hermosillo (0.45, 0.17 & 0.0) respectively. These observations are not compatible with the widely-held views of genetic homogenization of populations of highly migratory moth pest species, though overall mtDNA variability is lower than in many Diptera.

The allozyme studies on the above three Mexican and 12 US populations revealed both intra and intergroup genetic variability, especially, in low-frequency allozymes. Allelic frequencies at *Pgm*, *Adk*, *Ck*, and two *Est* loci can be used to estimate level of gene flow (migration). These studies will be completed by the end of 1993 and a comprehensive account of population genetic variation will be published. Similar mtDNA and allozyme studies on *H. virescens* are in progress.

INVESTIGATOR'S NAME(S): S. D. Pair

AFFILIATION & LOCATION: USDA, ARS, SCARL, Lane, OK 74555

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.4 Determine origins of adult populations

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Determined the parameters governing earworm population dynamics on corn in the LRGV, quantitated potential adult production, and defined the weather related transport mechanisms involved in the movement of populations into northern areas. Characterized the temporal sequence of population development with of movement in the LRGV, Uvalde, and Lubbock, TX. *Citrus* pollen was found on adult bollworm & budworm collected at Tifton, GA and at Lane, OK during April-May, 1991 indicating that the moths were not of local origin.

FY94 & FY95 WORK PLANS: Continue to maintain and monitor pheromone traps in support of ARS efforts to detect migratory events.

INVESTIGATOR'S NAME(S): J. R. Raulston[a], P. D. Lingren[b], K. R. Beerwinkle[b], J. D. Lopez[b] and S. D. Pair[c]

AFFILIATION & LOCATION: [a]USDA, ARS, CIRU, Weslaco, TX; [b]USDA, ARS, CIPMRU, College Station, TX; [c]USDA, ARS, SCARL, Lane, OK

ACTION AREA:	3.	Ecology and Populations Dynamics
LEAD ARRAY:	3.4	Determine origins of adult populations
OPTIM ARRAY:	3.4.2a	Identify and characterize source population zones
OPTIM ARRAY:	3.4.2b	Utilize standardized sampling procedures in multiple cropping systems across geographical regions to determine chronology of developing populations
OPTIM ARRAY:	3.4.2c	Determine regions in Mexico that may influence development of U.S.populations in the spring

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Population dynamics studies designed to determine the comparative chronology of population events were initiated in five corn growing areas of Texas and northeast Tamaulipas, Mexico including: Lower Rio Grande Valley, Uvalde, Wharton, Bell county, and Lubbock. These studies are designed to identify source and recipient zones of migrating corn earworm populations and to determine the impact of immigrants in recipient zones. In 1991 and 1992, the small larvae peak in whorl stage corn at LRGV occurred between 3/25-29; 22-35 days earlier than observed at Uvalde, Wharton or Bell Co. and 63 days earlier than Lubbock. Fifty percent silking at LRGV occurred 5/1-2; 15-26 days earlier than at Uvalde, Wharton or Bell Co. and 66 days earlier than Lubbock. The small larvae infestation peak on fruiting corn at LRGV occurred between 5/6-17, at Uvalde and Wharton between 5/20-31, at Bell Co. between 5/29-6/8 and at Lubbock between 7/8-26. Excavation of corn earworm pupae at LRGV averaged 1.7 and 0.37/m^2 in 1991 and 1992 respectively. Uvalde pupae populations were estimated at 3.6 and 2.8; Wharton at 3.3 and 0.4 ; Bell Co. at 3.3 and 0.8 ; and Lubbock at 3.7 and 2.1/m^2 for 1991 and 1992 respectively. Emergence peaks at LRGV occurred between 6/6-12 and 6/12-18 in 1991 and 1992 respectively. Emergence peaks at Uvalde, Wharton, Bell Co. and Lubbock occurred 16, 14, 26 and 76 days respectively after the peak at LRGV. Boll worm infestation peak on cotton occurred during or immediately after the emergence cycle in corn. Chronological differences in corn earworm populations are correlated to latitude phenology of the corn crop at the test locations. An increase of 1° latitude resulted in a 7-10 day delay in population development. Bollworm infestation peaks at all locations were synchronized with emergence from corn at the local level, indicating that cotton may not be a suitable host for determining when migratory influxes from other regions are occurring.

A trapping system has been installed in Mexico and a Memorandum of Understanding has been developed to aid in enlisting cooperation from scientists to provide trap data on a daily basis. These trap data will be used to identify regions in Mexico that may provide spring populations of corn earworm that migrate northward.

FY94 & FY95 WORK PLANS: Comparison of the population dynamics of *H. zea* in corn and cotton will be continued at the 5 listed locations. The data will be utilized to develop plant/insect models to provide prediction capabilities for the development of corn earworm populations within various regions based on latitude and crop phenologies. Magnitude of corn earworm populations will be estimated in all regions based on the excavation and emergence of pupae from local corn fields. These data will be used to determine the timing and magnitude of populations available for dispersal and migratory movement.

INVESTIGATOR'S NAME(S): R. L. Roehrdanz

AFFILIATION & LOCATION: USDA, ARS, BRL, Fargo, ND 58105

ACTION AREA: Ecology and Population Dynamics

LEAD ARRAY: 3.4 Determine origin of adult populations

OPTIM ARRAY: 3.4.2d Analyze genetic variation in natural populations

DATES COVERED BY REPORT: October 1991-June 1993

PROGRESS REPORT: Mitochondrial DNA restriction site variability was surveyed in geographically divergent collections of *H. virescens*. Individuals from four locations were used (Georgia, California, and two in Mexico). Two types of mtDNA analysis were employed. The standard technique included digesting total DNA with restriction enzymes and hybridizing Southern blots with a labelled purified mtDNA. Most of the same individuals were also tested for restriction site variation in two PCR-amplified portions of the mt genome, the 16S rDNA and the COI-COII regions. This latter approach was also used to test a smaller number of individuals from Tennessee, Mississippi, Oklahoma, and Weslaco (Texas), along with samples collected in April, June, July, and September in College Station (Texas).

For the total mtDNA, 11 restriction enzymes were used that produced about 65 sites. These sites sampled about 350 base pairs of DNA out of the 16,000 base pair total mtDNA. About 70 individuals from four locations were used (Tifton, Georgia; Brawley, California; Ciudad Obregon, Mex.; Hermosillo, Mex.). A restriction site map was also prepared for some of these sites. Most of the same individuals along with some additional insects were used for the PCR-based analysis. Seven restriction enzymes produced about 30 restriction sites and sampled about 175 base pairs of sequence.

Seven haplotypes were identified for the Southern blot data. Haplotype 1 (total) comprises 50/59 (85%) individuals. The other six haplotypes were found only once or twice. The PCR data also uncovered seven haplotypes. Haplotype 1 (PCR) was found in 118/131 (91%) individuals. The second most frequent haplotype comprised only 5% of the individuals. The other five haplotypes were found only once. Population distributions show that the rare haplotypes are scattered among the geographical locations.

MtDNA variability in *H. virescens* collections from diverse geographic locations is insufficient to warrant classification of the geographic collections as distinctive subpopulations. This would suggest that the overall population annually expands from a common reservoir population or that incoming migrants breed freely with those that may have overwintered locally. The findings are consistent with the reported migratory nature of the species, distribution of morphometric types in the western hemisphere, and a survey of enzymatic polymorphisms in the southern USA. No obvious seasonal differences were observed in the small number of individuals examined.

The results obtained from hybridization to total mtDNA and PCR amplification of selected regions lead to the same conclusion. Standard RFLP techniques have the drawback of being time consuming and limited by the amount of DNA that can be obtained from small individual insects. Complete sequencing of PCR amplified regions of mtDNA provides a wealth of information, but is both time consuming and expensive. Combining PCR amplification with RFLP analysis makes it possible to obtain data quickly from small samples of DNA and does not involve the use of radioactive isotopes.

INVESTIGATOR'S NAME(S): J. K. Westbrook

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.4 Determine origins of adult populations

OPTIM ARRAY: 3.4.2a Identify and characterize source population zones

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Several $1m^3$ and $2m^3$ tetrahedral-shaped, mylar balloons (tetroons) were launched from Weslaco (June 1992-1993), Eagle Lake (July 1992) and Plainview (August 1992), TX during emergence of adult corn earworm from commercial corn fields. The tetroons were ballasted to drift at altitudes of insect flight (approximately 700m above ground level), and launched nightly at the time of flight initiation (about 0.5h after sunset). Each instrumented tetroon was tracked for up to 9h by either the ARGOS satellite or a radio-equipped van. Four of the six tetroons tracked for one night from Weslaco in 1992 were located at a mean displacement of 299km $\pm$ 95km and direction of 339° $\pm$ 7°. A three-successive-night (27h) tetroon trajectory originated at Eagle Lake and passed near Atlanta, TX, Jonesboro, AR and Nashville, TN, on successive nights for a total displacement of 1394km. Seven tetroons were tracked from Plainview all night or until atmospheric subsidence or precipitation downed the tetroons. The tetroons displaced from 30km to 363km toward greater than 157° or less than 4°. Seven tetroons were tracked all night from Weslaco in 1993, and were located at a mean distance of 417km $\pm$ 91km and mean direction of 340° $\pm$ 13° from Weslaco. Further, two tetroons were launched simultaneously from two locations in the lower Rio Grande Valley on two nights, and separated by an increased distance of 85km (293%) with the easternmost tetroon displacing farther north to cause a 32° counterclockwise rotation of the segment joining the tetroon pair. Collective insect flight toward a fixed heading at 5 m/s could add up to 162km to these tetroon trajectories.

FY94 & FY95 WORK PLANS: Initial field studies will coincide with the citrus blooming period (mid-March through mid-April) in the lower Rio Grande Valley (LRGV) which provides a natural pollen mark to moths that feed on it. Other field studies will coincide with moth emergence from fruiting corn in the LRGV. Methods to internally mark newly emerged moths on a large scale will be investigated. Adults will be captured in the LRGV and anticipated fall-out areas to determine the number of marked moths that have exited and entered these areas, respectively. A stochastic compartmental model of population dispersal will be derived from the relationship between adults in compartments in the LRGV and fall-out areas. A radio-equipped van and the ARGOS satellite will track instrumented tetroons to provide atmospheric trajectory data representing displacement of diffusing moth "clouds" for long distances. Tetroon altitude will be based on the initial flight altitude of the moth "cloud" measured by entomological radars. Instrumentation will measure atmospheric pressure, temperature and humidity of the ambient air. Tracking will continue until dawn. Insect behavior that affects migration distance and dispersal will be measured with ground-based and airborne radars. Behavior such as take-off and ascent at the source, insect flight speed, insect orientation, vertical distribution (insect layering), and mean flight altitude are derived from radar data. The spatio-temporal dispersion of insects from corn will be measured with radar and aerial sampling during the period of emergence and dispersal from the mature crop. Personnel tracking the tetroons will notify radar operators and entomologists to coordinate the downwind deployment of monitoring crews during the nightly migration events to measure fall-out. Aerial sampling will identify species as well as correction factors for application to radar data if other species occur in significant quantity. Airborne radar measurements of horizontal insect distributions will be compared with insect dispersal estimated by tetroons to differentiate between atmospheric dispersal and active flight. Mark-capture of adults in the source and recipient areas will provide the model parameters for developing stochastic models of insect population growth and dispersal.

INVESTIGATOR'S NAME(S): W. W. Wolf

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and population dynamics

LEAD ARRAY: 3.4 Determine origins of adult populations.

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Entomologist have identified areas in and near the Lower Rio Grande Valley, Wharton, TX, Uvalde, TX and Plainview, Texas as origins of corn earworm adult populations. Radar verified that large numbers are produced in the Lower Rio Grande Valley, and near Plainview, however, infestations were low at Wharton and only plumes of insects from individual fields were evidence of unusual moth production in this area. No radar observations have been made near Uvalde, TX.

FY94 & FY95 WORK PLANS: Prepare proposal for deploying radars at other locations along Rio Grande River. This strategy may detect the over-flight from source areas in north central and northwest Mexico. Deployment would be based on wind direction during moth emergence (March and April), and would indicate the source of early season migration. This early season migration may be a key factor in initiating or increasing subsequent generations within the US. Early season migration will affect the redundancy necessary for any area-wide suppression program using noninsecticidal techniques.

INVESTIGATOR'S NAME(S): J. D. Lopez, Jr., and K. R. Beerwinkle

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX.

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.5 Determine impact of migrant populations in recipient regions.

OPTIM ARRAY: 3.5.2 Determine spatial and temporal patterns of populations.

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Trap capture data were analyzed from an agricultural area in Burleson and Brazos counties in Central Texas during 1990 and 1991 to determine the spatial and temporal patterns of catches of *Helicoverpa zea* and *Heliothis virescens*. Traps were spaced at about 3.2 km intervals lines radiating from the center of a study area with a diameter of about 48 km (1,830 km^2) in 1990. In 1991, the number of traps was reduced by half. *H. zea* catches were much higher than *H. virescens* during early season. Decreases in trap catches culminated in very low catches of *H. zea* in mid-August: however, these were followed by dramatic increases in late August and early September that persisted unit mid-October. These increased catches were attributed to immigration from northern areas because all these adults could not have produced locally. In contrast, low catches of *H. virescens*, which were initially observed in early April, continued to increase very gradually until July when numbers began increasing to the highest levels of the season from late August to mid-October. Initial captures in early April were synchronized with the period of expected *H. virescens* overwintering emergence. Additionally, the pattern of population build-up that was keyed on the period of rapid cotton fruiting, especially in irrigated cotton appear to indicate a local origin of this species. The spatial pattern of catches of both species indicated that *H. virescens* adult activity was significantly more concentrated in the cropped than the uncropped areas in mid-season than *H. zea*. Cotton, especially the intensively managed irrigated acreage appears to play a major role in the activity pattern of *H. virescens*. Experimental definition of these proposed factors on the population dynamics of both species will aid in the development of area-wide pest management approaches.

FY94 & FY95 WORK PLANS: Sex pheromone trapping for *H. zea* and *H. virescens* will be continued in the area to determine the seasonal adult activity patterns and relate these to local environmental factors to develop a better understanding of the factors influencing population dynamics of the two species.

INVESTIGATOR'S NAME(S): S. D. Pair

AFFILIATION & LOCATION: USDA, ARS, SCARL, Lane, OK

ACTION AREA: 3. Ecology & Population Dynamics

LEAD ARRAY: 3.5 Determine impact of migrant populations in recipient regions

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Compiled information from adult corn earworm source and recipient areas over several years which suggest substantial annual impact of migration from the LRGV upon the High Plains of Texas and adjacent states during June and July. Similarly, corn earworm produced from corn on the High Plains severely impacts local cotton during August and September.

FY94 & FY95 WORK PLANS: None.

INVESTIGATOR'S NAME(S): J.L. Willers, T.L. Wagner, R.L. Olson, M.R. Williams, R.A. Sequeira

AFFILIATION & LOCATION: USDA, ARS, CSRU, Mississippi State, MS

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.5.2 Determine spatial and temporal patterns of populations

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Field sampling using line-intercept and quadrant-variance methods indicates that a random distribution is the dispersion pattern of *Heliothis* eggs on cotton. These results are being used to develop a statistical rule-base component of WHIMS, called BESS (Bayesian Expert System for Sampling).

The line-intercept sampling scheme is a good, but labor intensive, method to sample populations. The method can be used to develop survival curves of both *Heliothis* and broadly defined categories of cotton fruit. Other attributes of cotton stands can also be obtained using this method. This sampling plan has been coded and incorporated into WHIMS under the name, CASA (Computer Assisted Stand Analysis).

FY94 & FY95 WORK PLANS: We will use the line-intercept method to validate the BESS protocol and refine estimates of larval feeding rates and survivorship, as well as survivorship of cotton fruit. We plan to explore the impact of larval survivorship and feeding rates through simulation modeling in conjunction with findings from the sampling plans. We will revise CASA so that it deals with multiple attributes of selected sample units and improve its data-entry facility.

INVESTIGATOR'S NAME(S): J. K. Westbrook

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.5 Determine impact of migrant populations

DATES COVERED BY REPORT: September 1993-June 1993

PROGRESS REPORT: Several $1m^3$ and $2m^3$ tetrahedral-shaped, mylar tetroons were launched from Weslaco (June 1992-1993), Eagle Lake (July 1992) and Plainview (August 1992), TX during emergence of adult *H. zea* from corn fields. The tetroons were ballasted to drift at altitudes of insect flight (about 700m AGL), and launched nightly at the time of flight initiation (about 0.5h after sunset). Each instrumented tetroon was tracked for up to 9h by either the ARGOS satellite or a radio-equipped van. Four of the six tetroons tracked for one night from Weslaco in 1992 were located at a mean displacement of 299km and direction of 339°. A three-successive-night (27h) tetroon trajectory originated at Eagle Lake and passed near Atlanta, TX, Jonesboro, AR and Nashville, TN, on successive nights for a total displacement of 1394km. Seven tetroons were tracked from Plainview all night or until atmospheric subsidence or precipitation downed them. They displaced from 30km to 363km toward greater than 157° or less than 4°. Seven tetroons were tracked all night from Weslaco in 1993, and were located at a mean distance of 417km and mean direction of 340° from Weslaco. Two tetroons were launched simultaneously from two location in the LRGV on two nights, and separated by an increased distance of 85km (293%) with the easternmost tetroon displacing farther north to cause a 32° counterclockwise rotation of the segment joining the tetroon pair. Collective insect flight toward a fixed heading at 5 m/s could add up to 162km to these tetroon trajectories.

FY94 & FY95 WORK PLANS: Initial activities will coincide with citrus blooming (mid-March through mid-April) in the LRGV. Citrus pollen provides a natural mark to moths that feed on it. A second study period will coincide with moth emergence from corn in the LRGV. Methods to internally mark emerging moths on a large scale will be investigated. Self-labeled adults will be captured in the LRGV and anticipated fall-out areas to determine the number of marked moths that have exited and entered these areas, respectively. A stochastic compartmental model of dispersal will be derived from the relationship between adults in compartments in the LRGV and fall-out areas. The model will specify transfer rates quantifying the number of adults migrating from the LRGV relative to the resident population in the fall-out areas. Independent sets of marked moths captured in fall-out areas and radar detection of movement to fall-out areas will be used to validate the model. A radio-equipped van and the ARGOS satellite will track tetroons to provide trajectory data representing the displacement of diffusing moth clouds for long distances. Instrumentation will measure atmospheric pressure, temperature and humidity of the ambient air. Moth take-off, ascent, flight speed, orientation, vertical distribution and flight altitude will be derived from radar data. The spatio-temporal dispersion will be measured by radar and aerial sampling. Personnel tracking tetroons, radar operators and entomologists will coordinate the downwind deployment of monitoring crews during the nightly migration events to measure fall-out. Aerial sampling will identify species as well as correction factors for application to radar data if other species occur in significant quantity. Airborne radar measurements of horizontal insect distributions will be compared with dispersal estimated by tetroons to differentiate between atmospheric dispersal and active flight.

INVESTIGATOR'S NAME(S): K.R. Beerwinkle[a], P.D. Lingren[a], J.D. Lopez, Jr.[a], P.G. Schleider[a], R.S. Eyster[a], and J.A. Witz[b]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b]Formerly USDA, ARS, PMRU, College Station,TX, currently, Texas Transportation Institute, Texas A&M University, College Station, TX

ACTION AREA: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.6 Migration technology

OPTIM ARRAY: 3.6.2a Characterize dispersal attributes of moth clouds arising from source areas

OPTIM ARRAY: 3.6.2b Develop and improve aerial sampling technology

SUPPL ARRAY: 3.6.3b Correlate meteorological parameters with insect transport during migration

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: An automated, vertical-looking x-band radar system was developed for continuously monitoring aerial insect activity (Beerwinkle et al. 1993). The system is comprised of a 10kW marine transceiver fitted to a 1.83-m diameter circular parabolic dish antenna that is equipped with a noise-attenuating shroud and a rotating feed. The display has seven-level signal detection circuitry which is interfaced with an IBM AT-compatible 386 PC to provide automatic counting of radar return echoes in seven amplitude ranges. Data provided by the system can be used to estimate a size distribution of detected targets. Calibration results indicate that noctuid-sized insect targets can be detected out to a maximum range of about 2500 m.

Research to develop and improve aerial sampling technology was not actively pursued during this reporting period; however, some aerial sampling was conducted in conjunction with entomological radar research of insect migration. Pairs conical nets (0.16 m^2 inlet) were towed with a Cessna 206 aircraft for a total of 6 hours at various altitudes from 150 to 600 m during 3 nights in late June, 1991, in Wharton County near Eagle Lake, TX. A total of five *H. zea* Moths (2 male, 2 female, and 1 whose sex could not be determined) were captured between 2110 and 2205 h on 25 June 1991 while sampling at 300 m AGL over a large corn field source area for emerging *H. zea*. Other limited aerial sampling was attempted with fixed-wing aircraft towing conical nets with inlets up to 1 m^2 in cross section in conjunction with entomological radar studies in the Lower Rio Grande River Valley and on the Texas High-Plains during the summer of 1992. However, aerial densities of noctuids were never sufficiently high for successful sampling.

FY94 & FY95 WORK PLANS: The vertical radar system will continue to be used in concert with other radar systems and meteorological support in our *Heliothis/Helicoverpa* spp. movement research. Research will also be conducted to improve aerial sampling technology to support the research on insect movement and migration.

INVESTIGATOR'S NAME(S): M. H. Greenstone

AFFILIATION & LOCATION: USDA, ARS, BCIRL, Columbia, MO

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.6 Migration technology

OPTIM ARRAY: 3.6.2b Develop and improve aerial sampling technology

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: An aerial sampling system comprising nets and rigging deployed on a slow-moving automobile in the surface boundary layer and on a slow-flying aircraft in the planetary boundary layer was developed and field tested. Preliminary sampling studies revealed the taxonomic and altitudinal distribution of natural enemy species in five orders of arthropod natural enemies: Araneae, Coleoptera, Hemiptera, Diptera, and Hymenoptera.

FY94 & FY95 WORK PLANS: The investigators role in arthropod natural enemy aerial dispersal will be reassessed as part of new CRIS development.

INVESTIGATOR'S NAME(S): J. K. Westbrook

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.6 Migration technology

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Several $1m^3$ and $2m^3$ tetrahedral-shaped, mylar balloons (tetroons) were launched from Weslaco (June 1992-1993), Eagle Lake (July 1992) and Plainview (August 1992), TX during emergence of adult corn earworm from commercial corn fields. The tetroons were ballasted to drift at altitudes of insect flight (approximately 700m above ground level), and launched nightly at the time of flight initiation (about 0.5h after sunset). Each instrumented tetroon was tracked for up to 9h by either the ARGOS satellite or a radio-equipped van. Four of the six tetroons tracked for one night from Weslaco in 1992 were located at a mean displacement of 299km $\pm$ 95km and direction of 339° $\pm$ 7°. A three-successive-night (27h) tetroon trajectory originated at Eagle Lake and passed near Atlanta, TX, Jonesboro, AR and Nashville, TN, on successive nights for a total displacement of 1394km. Seven tetroons were tracked from Plainview all night or until atmospheric subsidence or precipitation downed the tetroons. The tetroons displaced from 30km to 363km toward greater than 157° or less than 4°. Seven tetroons were tracked all night from Weslaco in 1993, and were located at a mean distance of 417km $\pm$ 91km and mean direction of 340° $\pm$ 13° from Weslaco. Further, two tetroons were launched simultaneously from two locations in the lower Rio Grande Valley on two nights, and separated by an increased distance of 85km (293%) with the easternmost tetroon displacing farther north to cause a 32° counterclockwise rotation of the segment joining the tetroon pair. Collective insect flight toward a fixed heading at 5 m/s could add up to 162km to these tetroon trajectories. We have evaluated National Weather Service NEXRAD doppler radar outputs to determine the appropriateness of this technology for insect migration research.

FY94 & FY95 WORK PLANS: Digital products from the NEXRAD, Profiler and other high-resolution remote sensing systems will be acquired and analyzed to determine the spatial redistribution of migrating insects and the nocturnal atmospheric environment in which they fly. Climatological and phenological data will be analyzed and spatially displayed using a Geographic Information System. Further, measured and simulated insect dispersal will be correlated with these environmental and biological distributions to better interpret the impacts of immigrants on local insect populations and crop production.

INVESTIGATOR'S NAME(S): W. W. Wolf

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and population dynamics

LEAD ARRAY: 3.6 Migration Technology

SAFEGD ARRAY: 3.6.1 Assess feasibility of doppler radar systems for migration research.

OPTIM ARRAY: 3.6.2a Characterize dispersal attributes of moth clouds arising from source areas.

SUPPL ARRAY: 3.6.3a Correlate meteorological parameters with insect transport during migration.

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: A WSR-88D (NEXRAD) doppler radar, located at League City, TX detected insects on the night of 19 July 1993. Mission requirements of these radars have resulted in software that prevents operating the radars in an optimum entomological mode. The clear-air mode is the only mode that has sensitivity for detecting low densities of insects. This mode restricts antenna elevations to 4.5 degrees or less and it takes 10 minutes for the antenna to sweep through 5 elevation increments (0.5, 1.5, 2.5, 3.5, & 4.5 degrees). Thus, events can only be observed every 10 minutes. Low elevation angles prevented resolving the depth of thin layers of insects. These radars detect insects to greater ranges but lack the resolution of conventional entomological radars. They provide a qualitative measure of insect distribution and the radial velocity of targets (toward or away from the radar). They could provide quantitative information if appropriate software and a method to determine the size distribution of the insects were developed.
Values of four parameters, derived from radar measurements, that characterize the ascent and departure of *H. zea* moths from corn fields in the LRGV are: (1) time of flight initiation = 30 min after sunset; (2) ascent rate = 1.5 m/s; (3) mean flight altitude = 404 m; and (4) insect-cloud-width = 43 km. These values represent the means during 1984 to 1990 for nights when the wind was from the SE.

Measurements of insect orientation at locations downwind of corn fields in NW Texas indicated that orientation changed with wind direction. Orientation resulted in displacement to the left of the wind displacement for 71% of the insect tracks examined (N = 239). A tracking radar was acquired to track individual insects.

FY94 & FY95 WORK PLANS: Determine insect flight behavior, measure insect flight duration, cloud displacement relative to wind displacement, and vertical distribution downwind of source areas. Develop insect classification technology.

INVESTIGATOR'S NAME(S): D. E. Hendricks

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.7 Optimize rbWHIMS model for cotton production and protection

OPTIM ARRAY: 3.7.1b Develop and integrate methods into WHIMS to deal with management uncertainties

DATES COVERED BY REPORT: January 1990-December 1991

PROGRESS REPORT: Flooding indigenous soils in which larvae of field-collected bollworms and tobacco budworms had pupated caused 100% mortality after five and four days, respectively. Time required for 50% (LT50) mortality of the bollworms was 62 hours, and LT50 for tobacco budworms was 50 hours. Larvae were collected from cultivated hosts (cotton and corn) or from native wild plant hosts, and soil was collected from the same locations. Alluvial loam soils used for these tests were types typically found in cotton-growing regions of the Mississippi and Rio Grande River deltas. Last stage (prepupae) larvae placed on dry soil in 1-liter glass jars were allowed four days to pupate after burrowing into the soil. The soil was then saturated by filling the jars with water. After continuous inundation for treatment periods ranging from 1 to 10 days, pupae were removed from the flooded soil, held in moist VermiculiteR and allowed time to emerge as moths, and percentage mortality was determined. Effects of seasonal flooding on survival of pupae in saturated soil near host crops can be incorporated into population prediction models such as rbWHIMS model for cotton production and protection.

FY94 & FY95 WORK PLANS: Supply mortality data from pupae flooding tests and seasonal data from standardized pheromone survey traps to validate insect population predictions made by computer models. Cooperators: J. L. Willers, T. L. Wagner, R. L. Olsen.

INVESTIGATOR'S NAME(S): T. L. Wagner, J. L. Willers, R. L. Olson, M. R. Williams, R. A. Sequeira, and R. O. Bowden

AFFILIATION & LOCATION: USDA-ARS, CSRU, Mississippi State, MS

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.7 Optimize rbWHIMS model for cotton production and protection

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Significant progress has been made on the development of GOSSYM/COMAX/WHIMS. The pest management component, rbWHIMS, was completely redesigned and rewritten. Originally coded in ART-IM, it is now written in C++ using an object-oriented design which more fully represents components of the agroecosystem. A new user interface was written in Microsoft Windows 3.1, improving the speed, reliability, and confidence of data entry into the system. The report generator, which provides a written record of scouting activity and system recommendations, has been improved significantly. New code was written for archiving input data into permanent files. These combined changes make the program more stable, flexible, maintainable, and usable.

rbWHIMS was thoroughly evaluated using field data collected in 1991 and 1992. Extensive use of the program permitted its verification. Numerous changes were made to the rule base as a result of this critique. Several new pest species were added--WHIMS now makes recommendations on cutworms, boll weevils, bollworm/budworms, early- and late-season thrips, early- and late-season plant bugs, spider mites, bandedwing whiteflies, aphids, yellowstriped, fall, and beet armyworms. Verification and validation of the 1993 version of WHIMS continues and field data are being collected from three Mississippi production farms for this purpose. Similar work on a small scale was arranged in Missouri, in co-operation with Dave Albers and Ray Nabors.

A mathematical programming problem was formulated and solved, which balances the costs of several management tactics against losses in profit due to feeding damage of larvae. This formulation provides a possible platform for risk assessment in the face of uncertainty for the WHIMS system.

FY94 & FY95 WORK PLANS: We will continue to revise the WHIMS' rule-base, specifically to deal with multiple pest species that occur concurrently, pest resistance problems, and time-dependent phenomena. A user's guide and documentation for WHIMS will be written, and the scouting protocol document revised. We will perform a rigorous statistical analysis on the output of the mathematical program mentioned above, while doing a sensitivity analysis of several parameters. The new formulation will be incorporated into BESS and WHIMS. Electronic pheromone traps will be assessed for real-time monitoring of moth flights.

INVESTIGATOR'S NAME(S): R. A. Sequeira, R. L. Olson, T. L. Wagner, J. L. Willers, and M. R. Williams

AFFILIATION & LOCATION: USDA, ARS, CSRU, Mississippi State, MS

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.8 Integrate WHIMS and GOSSYM/COMAX

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: rbWHIMS is presently being linked to GOSSYM/COMAX. Extant insect population models and an economic evaluation component (TEXCIM) are also being integrated into the system. A new structure to the data files is being developed to accommodate the integration. Conditional probability concepts are being utilized to reconcile conflicts between plant- and insect-induced mortality factors. This is the area where most other simulation models fall short in describing the dynamics between the plant and its pests. A novel statistical analysis method, based on the additive main effects and multiplicative interaction model, was developed to aid in the calibration and validation of the combined simulation system. These changes will greatly expand the power and utility of the system, although their full impact will not be realized for some time.

FY94 & FY95 WORK PLANS: We will complete the integration of all computer systems into one comprehensive package; after which components will be verified and validated.

INVESTIGATOR'S NAME(S): M. H. Greenstone

AFFILIATION & LOCATION: USDA, ARS, BCIRL, Columbia, MO

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.9 Develop optimum sampling procedures.

SAFEGD ARRAY: 3.9.1b Develop method to differentiate eggs and small larvae of *H. virescens* and *H. zea*.

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: The use of purified vitellin as immunogen led to a subfamily-specific (heliothinae) monoclonal antibody for egg identification. Use of whole homogenate as immunogen shows better promise for production of a species-specific antibody (3.9.1b, year 2).

FY94 & FY95 WORK PLANS: Development of monoclonal antibodies will continue, emphasizing cloning and adaptation of hybridoma cell lines to serum-free medium for antibody production in addition to specificity and sensitivity. Antibodies will be characterized as to species recognized, including *Helicoverpa armigera*, and as to egg and larval antigenic determinants recognized.

INVESTIGATOR'S NAME(S): D. E. Hendricks

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.9 Develop optimum sampling procedures

3.9.1a Improve capabilities of estimating pest abundance from limited sample data

DATES COVERED BY REPORT: January 1990-August 1992

PROGRESS REPORT: Field experiments were completed to determine the accuracy of an automatic system to detect bollworm and tobacco budworm moths attracted to baits made of their specific sex pheromones. The system included a telemetry system that sent a radio signal to a receiver and computer when a moth was detected at night. The computer was programmed to collate the number of moths detected from each detector unit installed in the field near host crops. Overall accuracy throughout a 6-month growing season was >98%, and accuracy ranged from 87% when numbers of moths detected or caught per night were high (>25) to 100% when numbers caught or counted per night were low (0 to 24). No significant differences were found between numbers of either of these species caught in traps equipped with or without detector systems.

FY94 & FY95 WORK PLANS: Link moth detector output to data loggers that can be interfaced via telephone lines to a computer. Cooperators, JLW, TLW, RLO, DEH.

INVESTIGATOR'S NAME(S): J. D. Lopez, Jr.[a], M. A. Latheef[b], J. A. Witz[c], P. D. Lingren[a], and J. R. Raulston[d]

AFFILIATION & LOCATION: [a]USDA, ARS, CIPMRU, College Station, TX; [b]USDA, ARS, AARU, College Station, TX; [c]Formerly USDA, ARS, PMRU, College Station, TX, currently Texas Transportation Institute, Texas A&M University, College Station, TX; [d]USDA, ARS, CIRU, Weslaco, TX

ACTION ARRAY: 3 Ecology and Population Dynamics

LEAD ARRAY: 3.9 Develop optimum sampling procedures

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Analyses of data from the Pilot Test "Area-wide Management of *H. zea* and *H. virescens* by Pheromone Trap Calibration" (1987-89), on captures of corn earworm in pheromone traps and during nocturnal sampling and the relationship to egg and adult densities in field corn indicated that: (1) percentage of nightly trap catch was highest between 2100 and 2200 h (CDT), (2) coefficient of determination between number of males caught in traps between 2100 and 2200 h and egg densities on the following morning (R^2=37) was higher than for other periods, (3) stepwise regression showed that trap catch between 0400 and 0500 h in combination with numbers of fresh silks per ha provided the best equation for predicting egg densities (R^2=51), (4) 62% of adults captured during nocturnal sampling were females with the highest percentage being caught between 2100 and 2400 h; male captures were nearly uniform throughout the night, (5) percentage of mating pairs was highest between 0300 and 0400 h, (6) temporal patterns of captures of males in the pheromone traps and mating pairs did not reflect competition between the traps and native females; there was no decrease in the fraction of males captures in pheromone traps when mating activity was highest and (7) there were significant linear regression relationships between corn earworm male capture per trap per night and density of females (R^2=67%) and males (R^2=69%) estimated from nocturnal sampling. An M.S. graduate student in Statistics is currently evaluating the relationship between captures of males in pheromone traps and density estimates from nocturnal sampling for corn earworm in cotton. This analysis is similar to that reported by Witz et al. (1992) for the tobacco budworm on cotton. Two sizes of malaise traps (1 m and 6 m) were evaluated for capturing corn earworm, tobacco budworm and other noctuids in an area containing dense stands of ergot-infected dallisgrass. Both trap sizes were effective in capturing corn earworm, tobacco budworm, black cutworm, beet armyworm, true armyworm, green cloverworm, cotton leafworm, variegated cutworm, fall armyworm, cabbage looper, soybean looper, and others. The efficiency of the two sizes of malaise traps was not proportional to the area indicating that other factors such as height of the traps may influence capture. In addition to monitoring adult activity of noctuids, the malaise traps have the potential to be used to bioassay the attractiveness of plants or chemicals. Emergence of *H. zea* from senescent corn was monitored by: (1) pupal sampling in 94 fields and placement of live pupae in two designs of individual emergence cages, (2) use of large screen emergence cages in selected fields, and (3) direct nocturnal observations from a corn plot. Differences were found in the emergence pattern and adult densities detected by the 3 techniques. The best technique will depend on the use of the emergence data, available resources, and specific aspects of the situation with the uniformity of corn phenological development being of critical importance.

FY94 & FY95 WORK PLANS: Analyses of pilot test data will be continued. Evaluations of malaise traps for monitoring the activity of corn earworm and other noctuids by trapping in areas containing plants that have been shown to attract noctuids will be continued. Malaise traps will be used to evaluate the response of corn earworm and other noctuids to different semiochemical concentrations represented by different numbers of attractive sources such as plants or chemical dispensers. Malaise traps of different heights will be evaluated to determine if efficiency can be increased.

INVESTIGATOR'S NAME(S): J. K. Westbrook

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.9 Develop optimum sampling procedures

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Several $1m^3$ and $2m^3$ tetrahedral-shaped, mylar balloons (tetroons) were launched from Weslaco (June 1992-1993), Eagle Lake (July 1992) and Plainview (August 1992), TX during emergence of adult corn earworm from commercial corn fields. The tetroons were ballasted to drift at altitudes of insect flight (approximately 700m above ground level), and launched nightly at the time of flight initiation (about 0.5h after sunset). Each instrumented tetroon was tracked for up to 9h by either the ARGOS satellite or a radio-equipped van. Four of the six tetroons tracked for one night from Weslaco in 1992 were located at a mean displacement of 299km $\pm$ 95km and direction of 339° $\pm$ 7°. A three-successive-night (27h) tetroon trajectory originated at Eagle Lake and passed near Atlanta, TX, Jonesboro, AR and Nashville, TN, on successive nights for a total displacement of 1394km. Seven tetroons were tracked from Plainview all night or until atmospheric subsidence or precipitation downed the tetroons. The tetroons displaced from 30km to 363km toward greater than 157° or less than 4°. Seven tetroons were tracked all night from Weslaco in 1993, and were located at a mean distance of 417km $\pm$ 91km and mean direction of 340° $\pm$ 13° from Weslaco. Further, two tetroons were launched simultaneously from two locations in the lower Rio Grande Valley on two nights, and separated by an increased distance of 85km (293%) with the easternmost tetroon displacing farther north to cause a 32° counterclockwise rotation of the segment joining the tetroon pair. Collective insect flight toward a fixed heading at 5 m/s could add up to 162km to these tetroon trajectories. The set of tetroon trajectories will indicate areas which should be most intensively surveyed to sample for immigrant adults downwind of source areas.

FY94 & FY95 WORK PLANS: Field activities will consist of two primary periods during each year of the study. The first period will coincide with the citrus blooming period (mid-March through mid-April) in the lower Rio Grande Valley (LRGV). Citrus pollen provides a natural mark to moths that feed on it. The second period coincides with most moth emergence from whorl- and fruiting-stage corn in the LRGV of Mexico and Texas. Food dyes or other types of markers will be investigated for internal marking of newly emerged adults. Emerging adults will be self-labeled by feeding on citrus in the LRGV and by markers previously applied to the soil or corn stalks in a 100 ha area of corn in the LRGV. Adults will be captured in the LRGV and anticipated fall-out areas to determine the number of marked moths that have exited and entered these areas, respectively. A radio-equipped van and the ARGOS satellite will track tetroons to provide atmospheric trajectory data representing displacement of diffusing moth "clouds" for long distances. The tetroon altitude will be based on the initial flight altitude of the moth "cloud" measured by entomological radars. Tracking of the tetroon will measure the atmospheric pressure, temperature and humidity of the ambient air, and the three-dimensional dispersal of an air parcel representing the center-of-mass of a passive moth "cloud." Tracking will continue until dawn. Insect behavior that affects migration distance and dispersal will be measured with ground-based and airborne radars. Behavior such as take-off and ascent at the source, insect flight speed, insect orientation, vertical distribution (insect layering), and mean flight altitude are derived from radar data. The spatio-temporal dispersion of insects from corn will be measured with radar and aerial sampling during the period of emergence and dispersal from the mature crop. Personnel tracking the tetroons will notify radar operators and entomologists to coordinate the downwind deployment of monitoring crews during the nightly migration events to measure fall-out. Aerial sampling will identify species as well as correction factors for application to radar data if other species occur in significant quantity. Airborne radar measurements of horizontal insect distributions will be compared with insect dispersal estimated by tetroons to differentiate between atmospheric dispersal and active flight. The location of tetroon displacements will aid in determining optimum insect trapping strategies.

INVESTIGATOR'S NAME(S): J. L. Willers, T. L. Wagner, R. L. Olson, R. A. Sequeira, and M. R. Williams

AFFILIATION & LOCATION: USDA-ARS, CSRU, Mississippi State, MS

ACTION AREA: 3. Ecology and Population Dynamics

LEAD ARRAY: 3.9 Develop optimum sampling procedures

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Refinement of the sampling protocol based on Bayesian concepts is still in progress. The most critical area of refinement involves delineating action thresholds, e.g., in relating the Bayesian estimates of population abundance to historical sampling methods. Observer error is still a difficult problem to overcome without the use of electronic aids (automated sampling aids are desperately needed to reduce the time and labor of sampling). The BESS (Bayesian Expert System for Sampling) prototype has been developed and currently is being coded into WHIMS under the Windows 3.1 operating environment. This system provides estimates of population abundance for cotton pest species. The estimates will be used to initialize WHIMS and the pest simulation models in the system.

FY94 & FY95 WORK PLANS: We plan to expand work into acoustical analysis of insect sounds associated with flight and feeding using digital signal processing techniques. We will develop a historical database to archive information for use in future years and to use in simulation studies for economic impact analyses on a field by field basis. Also, graphical methods will be developed within BESS to display scouting data as a function of time, both for the current and selected previous years.

TABLE 3. Summary of Research Progress for Action Area III, Ecology and Population Dynamics, in Relation to Year 2 Goals of the 5-Year Plan.

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
3.1 Quantify pre-flight activities.	Identify post-emergence preflight activity sites.	X		Moths emerging in senescing corn usually make only short flights on the night of emergence and leave the field on the night after emergence. Various types of non-obtrusive viewing systems have been identified for observing pre-flight activities but none have been procured. Detailed microscale weather data have been collected to determine the meteorological influences on pre-flight behavior, however these data have not been analyzed. Although some progress has been made in this array, it does not appear to be on track with the 5-year plan.
3.2 Determine adult response to plants and plant volatiles.	Develop procedures to qualify and quantify responses.	X		A 2- and 6-free choice olfactometer system has been developed and used to measure attractiveness of various plant volatiles. Both direct nocturnal observation and captures in Malaise traps showed field responses to ergot-infected dallisgrass and *Gaura suffulta* elicited strong proboscis extension response indicating the plant to be a feeding source. Oak, willow, Asteraceae, citrus and *Pithecellobium* pollens were found on *H. zea* indicating the attractiveness of these blooming plants. Velvetleaf was identified as an important spring and mid-season host for bollworm and tobacco budworm and a fall host for tobacco budworm in Mississippi. Two year goals have been met in this array.

TABLE 3 - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
3.3 Determine migratory and trivial flight initiation and termination.	Correlate differences between migratory and trivial flight with adult physiological differences.	X		Data were collected on population dynamics of H.zea and H. virescens in some major cropped areas of Texas and northern Mexico. Population events coincided with latitude and crop phenology. Pollen including *Citrus* was used to detect migration and analysis of transport opportunities identified potential source areas. Genetic analyses performed on *H. zea* males from various locations in the U.S. and Mexico have revealed both intra and intergroup variability in low frequency allozymes. Mitochondrial DNA variability in *H. virescens* collections from diverse locations in the U.S. and Mexico was insufficient to warrant classification of collections as distinctive subpopulations. Tracking data detailed in 3.3 are applicable to 3.4.
3.4 Determine origins of adult populations.	Establish procedures for overall study and recruit cooperators.	X		Estimates of displacement of *H. zea* were provided by tracking tetroon launched at time of flight initiation from source zones. Data on flight altitude and periodicity were obtained with radar. Real time digitization of airborne radar was developed for quantifying boundary layer flight. Effects of sex, mating status, light, temperature, relative humidity, rearing condiditons and allatectomy on flight of *H. zea* were evaluated with flight mills. Flight speed orientation and other behaviors were observed from ground observations of ascent and initial translation of *H. zea* moths.

TABLE 3 - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
3.5 Determine impact of migrant populations in recipient regions.	Collect data to determine synchrony of chronological events among populations and regions.	X		Area wide pheromone trapping in Central Texas verified earlier occurrence of *H. zea* in spring, contrary to expectations due to local emergence from diapause. *H. viresens* concentrated in cropped areas and return migration of *H. zea* in the fall was determined. Line intercept and quadrant-variance sampling methods indicated random dispersion of *Heliothis* eggs on cotton. Line intercept sampling has been incorporated into WHIMS. Spatial and temporal patterns of populations, were determined, however interregional aspects have not been emphasized.
3.6 Migration technology.	Assess and develop equipment for electronic identification of species undergions migratory flight.	X		Tracking of tetroons and tracking radar mentioned in 3.3 are applicable to 3.6. An automated vertical radar capable of estimating size distribution of targets to 2500 m was developed. Assessment of dopler radar indicated the equipment can provide qualitative measures of insect distribution and radial velocity of targets and could provide quantitative data with appropriate software. These radars detected insects to greater ranges but lacked resolution of conventional radar. Aerial sampling systems on automibiles and slow moving aircraft were developed and tested. Four dispersal attributes; flight initiation, ascent rate, flight altitude, and insect cloud width were determined with radar. Development of a climatological atlas is planned for 1994 & 1995.

TABLE 3 - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS N0	SIGNIFICANCE
3.7 Optimize reWHIMS model for cotton production and protection.	Research, develop, implement, and test system components.	X		rbWHIMS was redesigned to take advantage of object-oriented programming capability of C++ language. New interface with MS Windows. rbWHIMS was evaluated and adjusted with field data. Twelve new pest species were added to WHIMS recommendations.
3.8 Integrate WHIMS and GOSSYM/COMAX.	Develop WHIMS to predict impact of insect on host.	X		rbWHIMS is being linked to GOSSYM/COMAX. Existing population models and evaluation components are being integrated into the system. A novel statistical method was developed for calibration and validation of the simulation system. No progress or plans were reported relative to geographic information system.
3.9 Devleop optimum sampling procedures.	Design alternative sampling protocols and collect data.	X		Progress in 3.3 and 3.6 are applicable. A Bayesian Expert System for Sampling was developed and being encoded into WHIMS. Automated sampling aids are needed to reduce time of sampling. An automatic system for detecting moths responding to pheromone was >98% accurate. The relationship of egg and adult densities in corn was evaluated. Malaise traps were determined to have potential for monitoring adult activity. Use of population monitoring techniques including pupae sampling from many fields, placement of pupae in individual emergence cages, large emergence cages in selected fields and nocturnal observations were evaluated for determining the pattern and density of *H. zea* emergence from senescent corn. An immunogen using purified vitellin led to a subfamily-specific monoclonal antibody for egg identification.

RESEARCH SUMMARY: ACTION AREA III—ECOLOGY AND POPULATION DYNAMICS

Compiled by J. D. Lopez and T. Popham

LEAD ARRAY 3.1: Data have been collected, but not yet analyzed, on microscale weather at emergence sites and associated preflight behavior of *H. zea* in senescent corn. Nocturnal observation equipment especially a tracking radar (3.3 LEAD) is being evaluated. Plans are for instrumentation to determine profiles and gradients of atmospheric wind flow, temperature, and relative humidity in sweet corn and other field crops up to 100 m concurrent with night vision observations of adult behavior. Data collection goals of year 2 for preflight activities (3.1 LEAD) have been met. No progress has been made on determining influence of reproductive and feeding sites available to newly emerged adult populations (3.1.2a OPTIM). Plans are made for year 3, with plans unclear for years 4 and 5 on integration of findings into adult control systems.

LEAD ARRAY 3.2: A 2- and 6-free choice olfactometer system has been developed and used to measure attractiveness to adult *H. zea* of ergot-infected dallisgrass seedheads, flowers of 3 *Gaura* species, citrus, oak, willow, other flowering plants, and synthetic compounds identified from attractive plants. Both direct nocturnal observation and catches in Malaise traps showed the field response by *H. zea* and *H. virescens* to ergot-infected dallisgrass. *Gaura suffulta* nectar (30% dissolved solids) elicited strong proboscis extension from laboratory-reared and field-collected *H. zea*. Adult *H. zea* fed on apparently ergot-infected seedheads of a coarse rhizomatous grass. Pollen load analysis identified potential adult food plants. *Citrus* and *Pithecellobium* pollen were found on *H. zea* moths captured in Oklahoma. Oak, willows and Asteraceae were also found to be attractive to *H. zea* based on pollen contamination. Velvetleaf is an important spring and mid-season host for bollworm and tobacco budworm and a fall host for tobacco budworm in Mississippi. A tactile or chemical oviposition stimulant may be involved. Two year goals are met and plans for years 3 and 4 conform, except identification/isolation of possible adult attractants (year 5) is early.

LEAD ARRAY 3.3: Estimates of displacement of *H. zea* were provided by tracking mylar balloons (tetroons) launched at time of flight initiation from source zones. Data on daily and seasonal periodicities of nocturnal flight and altitudinal distributions were obtained with radar. Real time digitization of airborne radar was developed for quantifying boundary layer flight. Tracking radar capable of tracking individual *H. zea* to 1.3 km was acquired. Effects of sex, mating status, light, temperature, relative humidity, rearing conditions and allatectomy on flight of *H. zea* were evaluated with computer controlled flight mills. Several *H. zea* age groups showed a spiral-like flight up to about 50 m and then a relatively direct line orientation when observed from ground level up to 100 m AGL. Flight speeds, displacement vectors, etc. were calculated for these flights. The spiral-like flight suggests the use of wind in initial moth orientation.Goals for year 2 were met and plans for years 3 and 4 conform.

LEAD ARRAY 3.4: Tracking data mentioned in (3.3) are applicable to (3.4). Data were collected on population dynamics of *H. zea* and *H. virescens* in some major cropped areas of Texas and northern Mexico. Pollen especially *Citrus* was used to detect migration and in conjunction with upper air transport opportunities to identify potential source areas. Genetic analyses were done of captured males from locations in U.S. and Mexico. Genetic analysis is apparently ahead of schedule. No progress was made on development of marking techniques at origin except for use of pollen; however, there are plans to investigate food dyes or other markers for internal marking of newly-emerged adults.

LEAD ARRAY 3.5: Tracking data mentioned in (3.3) are applicable to (3.5). Area-wide sex pheromone trapping of *H. zea* and *H. virescens* in Brazos River Valley in Central Texas verified earlier occurrence of *H. zea* in spring, contrary to expectations due to local emergence from overwintering, greater concentrations of *H. virescens* in cropped areas, especially irrigated cotton, and importance in reestablishment of *H. zea* populations by return migration from the north associated with fall cold fronts. A statistical rule-base component of WHIMS is being developed from results of line intercept and quadrant-variance methods which indicated random dispersion of *Heliothis* eggs on cotton. Line-intercept sampling method has been incorporated into WHIMS. Although labor intensive, it is a reliable sampling method which can be used to gather data for developing survival curves of *Heliothis* and broadly defined categories of cotton fruit. Progress has been made in

determining spatial and temporal patterns of populations (3.5.2 OPTIM). However, interregional aspects have not been emphasized, resulting in a one to two year lag.

LEAD ARRAY 3.6: Tracking of tetroons and the tracking radar mentioned in (3.3) are applicable to (3.6). An automated vertical radar system was developed for continuously monitoring aerial insect activity. Data from the system is capable of estimating size distribution of detected targets to a maximum range of about 2500 m. Mission requirements of doppler radar have resulted in software which prevents operating in an optimum entomological mode (3.6.1 SAFEGD). Four dispersal attributes have been characterized from observations during 1984 to 1990 when wind was from SE in Lower Rio Grande Valley (LRGV): (1) time of flight initiation = 30 min after sunset; (2) ascent rate = 1.5 m/s; (3) mean flight altitude = 404 m; (4) insect-cloud width = 43 km (3.6.2a OPTIM). In NW Texas observations indicated that orientation changes with wind direction resulting in displacement to the left of the wind for 71% of the 239 tracked insects (3.6.3a SUPPL). Aerial sampling systems deployed on slow moving automobile and slow moving aircraft were developed and field tested. Preliminary results are taxonomic and altitudinal distribution of natural enemies in 5 orders of arthropods. Aerial sampling in Wharton Co. TX for 6 hours during 1992 captured 5 *H. zea*, while other aerial sampling indicated aerial densities were not high enough for success. Assessment of doppler radar, development of aerial sampling has indicated problems resulting in a 1 year lag. Development of a climatological atlas is in plans for 1994 & 1995. Otherwise, goals are met.

LEAD ARRAY 3.7: rbWHIMS has been redesigned and rewritten to take advantage of object-oriented programming capability of C++ language. New interface with Microsoft Windows 3.1. rbWHIMS was evaluated with field data collected in 1991 & 1992 and adjustments made. Twelve new pest species added to WHIMS recommendations. Progress and plans conform to goals.

LEAD ARRAY 3.8: rbWHIMS is being linked to GOSSYM/COMAX. Existing insect population models and an economic evaluation component are being integrated into system. Conditional probability concepts are used to reconcile conflicts between plant and insect-induced mortality factors. A novel statistical method was developed for calibration and validation of the combined simulation system. Year 2 goals are met for integrating WHIMS and GOSSYM/COMAX. No progress or plans reported relative to geographic information system.

LEAD ARRAY 3.9: Progress mentioned in (3.3) and (3.6) are applicable to (3.9). Bayesian Expert System for Sampling (BESS) prototype developed and being coded into WHIMS. Observer error is a problem in refinement which might be overcome with electronic aids. Automated sampling aids are needed to reduce time and labor of sampling. Progress has been made in refining Bayesian sampling protocols. An automatic system to detect *H. zea* and *H. virescens* responding to sex pheromone baits had an overall accuracy > 98%. Analyses of 1987-89 Pilot Test data on *H. zea* captures in pheromone traps, nocturnal adult sampling and the relationship to egg and adult densities in corn provided important information. Efficacy of 1 and 6 m malaise traps in capturing *H. zea* and *H. virescens* in a dense stand of ergot-infected dallisgrass was not proportional to size, but the potential for monitoring adult activity was demonstrated. Pupal sampling in many fields and placement of pupae collected in individual emergence cages, large emergence cages in selected fields, and nocturnal observations from a plot were evaluated for determining the pattern and density of *H. zea* emergence from senescent corn. An immunogen using purified vitellin led to a subfamily-specific (Heliothinae) monoclonal antibody for egg identification. Use of whole homogenate shows better promise for production of a species-specific antibody. Progress and plans conform to the plan.

BREAKOUT SESSION SUMMARY

Several key points were identified during discussion sessions of the Ecology and Population Dynamics Action Area. One of the major points was that although this action area does not in itself provide a means of managing or controlling *Heliothis/Helicoverpa* populations on an area-wide basis, it does provide the context in which all the management approaches developed under the other action areas will be applied and evaluated. Successful implementation of these management approaches will depend on a thorough understanding of relative aspects of ecology and population dynamics. It was stressed during discussions that the biology and ecology of the system should lead the way in development of area-wide management. An improved understanding of the ecology and

population dynamics of the target species is critical for effective area-wide management and maintenance of strong research programs in this action area is required.

Migration is an important research thrust in this action area. Research emphasis is on *Helicoverpa zea* and significant progress is being made. The problem of *Heliothis virescens* control resulting from insecticide resistance and the apparent effectiveness of backcross sterility and application of NPV as mean of area-wide management indicate the need for expansion of research efforts on *H. virescens* migration. What influence does migration have on the development of insecticide resistance in different geographical and/or different crop areas and how does migration affect insecticide resistance management programs? An increase in insecticide resistance has been reported to be associated with a loss of fitness. How does this influence reproduction and dispersal of *H. virescens*? Because the ratio of native to released moths and build-up of the sterility trait in *H. virescens* populations could be greatly influenced by migration, it is important for the successful implementation of a backcross sterility management approach to have a better understanding of the role of migration in the build-up of local populations of *H. virescens*. Area-wide NPV treatment of wild geranium and effective control of populations during early season in the Mississippi Delta could be negated by in-season immigration of *H. virescens*. Initially, research might include pollen analyses of early-season sex pheromone-trapped males to determine if patterns similar to *H. zea* can be identified for *H. virescens*. The feasibility of including *H. virescens* in ongoing research on *H. zea* migration, particularly the IPM funded pilot test program, can be considered.

Interaction of adult insects with plant populations is another important research emphasis in this area. Much of the current research effort is directed at development of adult control technology, primarily through use of plant-source feeding attractants/stimulants. Although this research is relevant to the Host Plant Resistance and the Behavior Modifying Chemicals action areas, more emphasis could be given to the effects of host plants on adult reproduction, i.e. mating and oviposition. Research results indicate a strong influence of the host plant on various aspects of adult behavior which may affect migration and reproduction, knowledge of which are important to area-wide management. This research could provide a basis for the identification of female attractants which are needed for adult sampling which is basic to modeling population reproduction and movement. Sex pheromone trapping is very effective for trapping males for monitoring and sampling, so much of the research is biased toward males with an implicit assumption that the results are equally applicable to females. We need to know what sex ratios exist in reproducing and migrating populations to improve our understanding of the relationship of male captures to female densities. Also, behavioral sex differences need to be assessed. Recent findings of genetic influences on rate of reproductive development in *Heliothis armigera* independent of environmental cues indicate the need for similar investigations with *H. virescens* and *H. zea*. The importance of using field-collected adults or adults with limited time in laboratory culture must be emphasized.

Population modeling is another important aspect of this area. Historically, the effort has been directed at field level management. To insure compatibility of the modeling effort with the area-wide management goals will require modification of approaches and objectives of modeling research to include processes which have their effect on populations spread over wide geographic areas. Key processes would be intra-area dispersal, immigration, emigration, cultivated and wild host plant effects, and climatic and meteorological influences on spatial and temporal distributions and densities over diverse areas; coupled with the methods and technology to assess and predict these. An IPM pilot project on *H. zea* migration currently underway is apparently partially directed at these objectives. Should this effort include *H. virescens*? How compatible is this effort with the ARS work at Mississippi State, MS, from the standpoint of area-wide management? Does this work on *H. zea* represent a departure toward a requirement for modeling more compatible with area-wide management? If this is a movement toward broad area modeling, an important aspect will be the need for a Geographical Information System (GIS) compatible with the developing modeling system capable of real-time integration of sampled population information and projections based on modeling of the previously mentioned influences on target insect behavior. The GIS for boll weevil being developed at Mississippi State could be useful for *Heliothis/Helicoverpa* and its potential needs to be explored.

Accurate, unbiased, low cost, and rapid sampling on an area-wide basis is critical to other components of this and the other action areas. It is essential for monitoring populations as well as assessing effects of area-wide management approaches. No consensus exists concerning how *Heliothis/Helicoverpa* should be sampled. Should

sampling be directed at immature or adult stages? The resources required for different stages can be very different. If immature stages are sampled, how will the population information relate to adults, reproduction and environmental influences. The same situation results if only adults are sampled. After much research on sex pheromone-baited trapping, it is still unclear what capture results mean relative to adult sex ratios, densities and distribution over time and space. There is a need for a sampling method not biased to either sex, if for no other reason then to give some meaning to male captures in sex pheromone traps relative to sex ratios and total population densities and distributions. An attractant which attracts females would be a major accomplishment and a research priority. Other methods without sex bias should be evaluated to determine their potential for adult sampling. Of all the areas discussed, this may be the most important to the other parts of this and other action areas. Because it is complex, it may be that is has been avoided. The potential for all ideas for improved sampling methodology related to area-wide management should be considered.

Action Area IV. BEHAVIOR-MODIFYING CHEMICALS

Coordinators: J. R. McLaughlin and A. K. Raina

INVESTIGATOR'S NAME(S): D. E. Hendricks

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 4. Behavior Modifying Chemicals

LEAD ARRAY: 4.1 Develop and implement methods to manage *Heliothis/Helicoverpa* populations in cropping systems with plant derived allelochemicals

SAFEGD ARRAY: 4.1.1 Develop oviposition attractants and stimulants as tools for monitoring Heliothis/Helicoverpa

DATES COVERED BY REPORT: January 1992-August 1993

PROGRESS REPORT: Velvetleaf, *Abutilon theophrastii* Medikus, and cotton were inspected for eggs and larvae of bollworms and tobacco budworms in both 1991 and 1992 from April through November. Velvetleaf supported major peaks of eggs of both species during the cotton-growing season and substantial tobacco budworm larval populations from late September to November of both 1991 and 1992. Eighty percent (80%) of the eggs found on velvetleaf during the sampling period of both years were found at the top of young milk-stage fruit (seed pods); this indicated that a tactile or/and chemical oviposition stimulant may be present at the top of young velvetleaf fruit. The remaining 20% of the eggs were found on terminal or young foliage and on bracts of flowers. In 1991, blooms and buds of this weed provided attractive feeding and oviposition sites for females of both species from about 30 days before to 40 days after peak blooming of cotton. Greater numbers of bollworm eggs were found on velvetleaf in June of 1992 than during the same period in 1991.

FY94 & FY95 WORK PLANS: Surveys and inspections of velvetleaf and other wild host plans will continue to determine what particular plant parts are commonly used for oviposition by bollworms and/or budworms. Extracts for chemical stimulants can be made from these parts and bioassayed during short periods of the season when blooms of cotton or soybeans are not present.

INVESTIGATOR'S NAME(S): D. M. Jackson

AFFILIATION & LOCATION: USDA, ARS, CRL, Oxford, NC

ACTION AREA: 4. Behavior Modifying Chemicals

LEAD ARRAY: 4.1 Develop and implement methods to manage *Heliothis/Helicoverpa* ssp. populations in cropping systems with plant-derived allelochemicals

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: The use of *Nicotiana kawakamii* as a trap crop for protecting flue-cured tobacco from infestations of tobacco budworms and hornworms was tested in replicated (3) field plots in 1992 & 1993 at Oxford, NC. Early in the season, *N. kawakamii* was heavily infested in all fields, whereas the tobacco plots (both adjacent and remote) were lightly infested.

The use of tobacco flowers as a lure for capturing tobacco pests and beneficials was tested using standard "Texas-style" wire-cone traps. *Heliothis virescens*, *Helicoverpa zea*, *Manduca sexta*, and *M. quinquemaculata* moths were captured during the two years of this experiment. Four flower-baited traps and four control (unbaited) traps were monitored three times a week during each summer season. Flowers were replaced daily. In 1991 total captures in flower-baited traps were: 48 male and 50 female tobacco budworm moths, 49 male and 18 female corn earworm moths, 146 male and 34 female tobacco hornworm moths, and 29 male and 29 female tomato hornworm moths. Several other species were caught in lesser numbers.

Over the past 12 years, native populations of tobacco budworms, corn earworms, and hornworm moths have been monitored in black light and pheromone-baited traps in Granville County, NC and Tift County, GA.

INVESTIGATOR'S NAME(S): D. M. Light

AFFILIATION & LOCATION: USDA, ARS, WRRC, PWA, Albany, CA

ACTION AREA: 4. Behavior Modifying Chemicals

LEAD ARRAY: 4.1 Develop and implement methods to manage *Heliothis/Helicoverpa* populations in cropping systems with plant derived allelochemicals

SAFEGD ARRAY: 4.1.1 Develop oviposition attractants and stimulants as tools for monitoring *Heliothis/Helicoverpa*

OPTIM ARRAY: 4.1.2 Combine oviposition attractants with sex pheromone to monitor and/or manage both sexes

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Have discovered that similar host-plant volatiles will synergistically enhance the pheromone capture efficiency of additional 14 diverse species of Lepidoptera.

FY94 & FY95 WORK PLANS: Electrophysiological studies will be undertaken to determine the olfactory receptivity/sensitivity of *Helicoverpa zea* to host-plant volatiles and to *Gaura* blossom volatiles.

INVESTIGATOR'S NAME(S): S. D. Pair

AFFILIATION & LOCATION: USDA, ARS, SCARL, Lane, OK

ACTION AREA: 4. Behavior Modifying Chemicals

LEAD ARRAY: 4.1 Develop and implement methods to manage *Heliothis/Helicoverpa* populations in cropping systems with plant derived allelochemicals

DATES COVERED BY REPORT: September 1992-June 1993

PROGRESS REPORT: Neem oil and crude extracts of a mint species were tested as potential oviposition deterrents when applied to silking sweet corn. Although limited effects were demonstrated with mint, in some cases the use of neem resulted in significantly greater numbers of eggs compared to untreated plots.

FY94 & FY95 WORK PLANS: Continue search for and testing of candidate plant species having potential as ovipositional deterrents to earworm and other noctuids attacking vegetables.

INVESTIGATOR'S NAME(S): F. C. Tingle, E. R. Mitchell, and R. R. Heath

AFFILIATION & LOCATION: USDA, ARS, IABBBRL, Gainesville, FL

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.1 Develop and implement methods to manage *Heliothis/Helicoverpa* populations in cropping systems with plant derived allelochemicals

DATES COVERED BY REPORT: January 1990-December 1992

PROGRESS REPORT: Assessing *Heliothis virescens*, reactions to isolated plant odors is necessary for identifingy and producing the attractive phytochemicals. Most attractant chemicals identified have been sex-specific pheromones, primarily produced by females and attractive to males. Identification of plant constituents that attract *H. virescens* adults, especially females, would expand opportunities to forecast potential damage levels in crops and in developing manipulative schemes such as attracticide baits for control of this pest. The behavioral response of tobacco budworm moths to volatiles from extracts of their host plants was observed in flight tunnel bioassays. When moths were released individually into a wind tunnel, most of them began to fly randomly in an upwind direction. Moths that detected the odor plume from the plant extracts flew upwind in a zigzag pattern toward the source dispenser. The moths that landed usually examined the substrates from which the odor was emitted by probing with their antennae, proboscis, and/or ovipositor. Mated female and virgin female and male *H. virescens* moths demonstrated host-finding, oviposition, and feeding-like behaviors to volatiles from extracts of cotton, susceptible tobacco, and a wild host, *Desmodium tortuosum*. Mated females laid significantly more eggs on substrates treated with extract from susceptible tobacco that on substrates treated with extract from a resistant variety.

Volatiles from extracts of cotton squares (flower buds), flowers, flowers without bracts, and petals from flowers attracted significantly more mated and virgin female and male *H. virescens* moths to extract-treated substrates than did corresponding controls. More mated females than virgin females or males landed on the treated substrates. We do not know if the same chemical components are present in cotton leaves, squares, and flowers, or if all the components exist in varying ratios throughout the plant.

FY94 & FY95 WORK PLANS: As promising as this line of research is, it has come to a complete halt. The chemical expertise needed to continue the research has been diverted to other projects.

INVESTIGATOR'S NAME(S): J. K. Westbrook, K. R. Beerwinkle, P. D. Lingren, and T. N. Shaver

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.1 Develop and implement methods to manage *Heliothis/Helicoverpa* populations in cropping systems with plant derived allelochemicals

DATES COVERED BY REPORT: September 1992-June 1993

PROGRESS REPORT: An automated weather station has been deployed in the center of a 20m x 20m plot of sweet corn. The weather station is instrumented with anemometers and wind vanes at several heights from 0.43m to 3.20m above ground level to measure the vertical profile of wind velocity within the developing corn canopy. Further, a three-dimensional propeller anemometer and three hot film anemometers are included among the sensors to measure high-frequency turbulence. The weather station in the center of the field and one at 2m outside the prevailing upwind perimeter of the plot are instrumented with air temperature, soil temperature, relative humidity and soil moisture sensors. Atmospheric dispersion values will be calculated to determine the amount of lateral and vertical mixing that could disperse plant derived allelochemicals above and under the corn canopy.

FY94 & FY95 WORK PLANS: Automated weather stations instrumented with several anemomemters and wind vanes at various heights from near ground level to 10 m will be deployed in a 40m x 40m plots of sweet corn and other field crops. The weather stations will be located at three locations within the plot to establish lateral gradients of the atmospheric wind flow and dispersion. An electrostatic air filter will be modified to ionize a known rate of air molecules. Five bipolar ion collectors will be constructed and attached to vacuum pumps. The ion collectors will be deployed in an orthogonal x-pattern (20m x 20m) with one axis parallel to the rows of corn, and will detect atmospheric ions as tracers of dispersing plant derived allelochemicals. Quantification and modeling of the concentration of dispersing ions will establish the high-frequency spatial distribution of neutrally-buoyant substances, and can be modified to account for differences in density and other relevant physical properties of various plant derived allelochemicals. Mean dispersal rates of various plant derived volatiles will be measured by vacuum sampling of air through an adsorbing substrate for several hours at five locations in the field plots and analyzing the concentration of the allelochemicals by gas chromatography/ mass spectrometry. Vaporization rates of various allelochemicals from several substrates used as lures will be measured in environmental chambers under controlled temperature, relative humidity and wind speed conditions. Subsequently, we will observe insect behavioral response to dispersing allelochemicals in the field plots using night vision equipment.

INVESTIGATOR'S NAME(S):		D. E. Hendricks
AFFILIATION & LOCATION:		USDA, ARS, SFCIML, Stoneville, MS
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.2	Develop and implement methods to suppress *Heliothis/Helicoverpa* populations with attracticide (allelochemical) baits for adults
OPTIM ARRAY:	4.2.2a	Select effective chemical toxicant and formulation for adults that do not interfere with the efficiency of the attractant

DATES COVERED BY REPORT: January 1992-August 1993

PROGRESS REPORT: No optimal formulation of toxicant and attractive bait is yet available for testing in field plots or broadly separated geographic areas.

FY94 & FY95 WORK PLANS: Formulations of attracticides can be evaluated in local field conditions when they become available. Attracticides can be compared with conventional insect control or monitoring approaches when they become available in quantities large enough to use in field plots.

INVESTIGATOR'S NAME(S):		D. M. Light
AFFILIATION & LOCATION:		USDA, ARS, WRRC, PWA, Albany, CA
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.2	Develop and implement methods to suppress *Heliothis/Helicoverpa* populations with attracticide (allelochemical baits for adults
SAFEGD ARRAY:	4.2.1	Determine whether plant-derived kairomone attractants enhance the efficiency of pheromone traps for capturing *Heliothis/Helicoverpa*
OPTIM ARRAY:	4.2.2b	Develop lab bioassay protocol for testing phytoattractants

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Blossom volatile composition of *Gaura* species being further analyzed, see Teranishi et al. Have discovered 15 host-plant volatiles that synergistically and preferentially enhance the capture efficiency (total capture) of *Helicoverpa zea* in double-cone traps (Scentry) baited with commercial pheromonal lures. Two host-plant volatiles have also been identified as pheromone synergists for *Heliothis virescens*.

FY94 & FY95 WORK PLANS: Field, night-vision behavioral studies will continue to determine the mode of action of these pheromone synergists.

Electrophysiological studies will also be undertaken to determine the olfactory receptor mode of action for this synergism.

INVESTIGATOR'S NAME(S): J. D. Lopez, Jr., P. D. Lingren, and T. N. Shaver

AFFILIATION & LOCATION: USDA, ARS, CIPMRU, College Station, TX

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.2 Develop and implement methods to suppress *Heliothis/Helicoverpa* populations with attracticide allelochemical baits for adults

OPTIM ARRAY: 4.2.2 Select effective chemical toxicants and formulations for adults that do not interfere with the efficiency of the attractant

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: The feeding time and volume and the proboscis extension response for unfed laboratory-reared 1-, 2-, and 3-day old male and female *H. zea* to the lowest recommended field rates of six commercial phagostimulants (Coax®, Gusto®, Entice®, Konsume®, Mo-Bait®, and Pheast®) was compared to the response when the adults were fed deionized water or 5% sucrose. With few exceptions for feeding time and with no exceptions for feeding volume, the response to the phagostimulants was not significantly different from the response to water alone and was significantly lower than the response to 5% sucrose. Subsequent feeding on 5% sucrose after feeding on the phagostimulants was high indicating that the moths didn't feed to satiation. A significantly greater percentage of one-day old moths exhibited a positive proboscis extension response upon tarsal contact with 5% sucrose than for the other treatments but the response to all treatments of two-day old moths was equal indicating a loss of discrimination as "nonspecific hunger" increased with age. Overall, the results indicated that the commercial phagostimulants are not effective adult feeding stimulants at the rates evaluated, but that the loss of discrimination for nonspecific substances with age due to nonspecific hunger and subsequent feeding on a highly stimulatory solution (5% sucrose) even after feeding on nonspecific substances are compatible with the use of effective feeding stimulants for adult control. Adult male and female laboratory-reared *H. zea* were able to feed on powdered or finely ground sucrose based on studies with radioactive sucrose. The adults "regurgitated" liquid through the proboscis that dissolved the sucrose and allowed the solution to be drawn up through the proboscis. Radioassays of adults fed a radioactive sucrose solution followed by nonradioactive solid sucrose showed that the source of the "regurgitant" is not the crop because no radioactivity was found in the powdered or ground solid sucrose after feeding. The adults also responded with a positive proboscis extension response upon tarsal contact with powdered or finely ground sucrose, thus showing the potential for a feeding response to dry materials in the field. Preliminary tests indicated that small crystals were necessary for response. The proboscis extension response to serial dilutions of sucrose, fructose, and glucose of laboratory-reared and field-collected (collected as pupae and emerged in a field cage, collected directly from fruiting corn, or captured in pheromone traps) *H. zea* was evaluated. The response of laboratory-reared adults to the three sugars was very consistent and was highest for sucrose > fructose > glucose. The response of field-collected moths to the three sugars was very variable and much lower than for the laboratory-reared moths. These results support the need for additional evaluations of field-collected moths for a better understanding of the factors influencing the response to sugar-based feeding stimulants for use in adult control technology.

FY94 & FY95 WORK PLANS: The feeding response to different sugars will continue to be evaluated in the laboratory to identify the optimum sugars and their concentrations as feeding stimulants for adult *H. zea*. The factors influencing the feeding response (of the adults) to sugars will also be evaluated to identify criteria for their use in adult control. Different formulations of the optimum sugar solutions will be evaluated for feeding stimulation in field cages and in the field. Evaluation of toxicants in conjunction with optimum feeding stimulants will be undertaken after effective feeding stimulant formulations are developed.

INVESTIGATOR'S NAME(S):		S. D. Pair
AFFILIATION & LOCATION:		USDA, ARS, SCARL, Lane, OK
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.2	Develop and implement methods to suppress *Heliothis/Helicoverpa* population with attracticide (allelochemical) baits for adults
DATES COVERED BY REPORT:		September 1992-June 1993

PROGRESS REPORT: A mixture of compounds identified from flowers of a vine was similar to bouquets from the same plant in its attractancy to *Heliothis/Helicoverpa* and other lepidopteran species.

FY94 & FY95 WORK PLANS: Continue efforts to identify active compound(s) attractive to adults for feeding. Implement additional studies to expand knowledge of insect species utilizing and/or responding to volatiles from plants demonstrating attractiveness for feeding.

INVESTIGATOR'S NAME(S):		T. N. Shaver, P. D. Lingren, and K. R. Beerwinkle
AFFILIATION & LOCATION:		USDA, ARS, CIPMRU, College Station, TX
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.2	Develop and implement methods to suppress *Heliothis/Helicoverpa* populations with attracticide (allelochemical) baits for adults.
OPTIM ARRAY:	4.2.2a	Select effective chemical toxicant and formulation for adults that do not interfere with the efficiency of the attractant.
DATES COVERED BY REPORT:		September 1991-July 1993.

PROGRESS REPORT: Extracts, headspace volatile collections and vacuum volatile collections were made of flowers of ten species of plants that have been demonstrated as attractive to *Helicoverpa zea* and other noctuids. Eleven compounds have been identified that are common to orange and grapefruit blossoms. An additional five compounds ave been identified from orange that were not present in grapefruit. Twenty-two other compounds have been tentatively identified from orange blossoms, eight of which were also contained in grapefruit. Several mixtures of the chemicals identified from hexane extracts of *Gaura* species have some biological activity to *H. zea* in laboratory olfactometer tests. Also, some of the headspace volatile collections of *Gaura* species have stimulated directed flight and proboscis extension in a wind tunnel using *Helicoverpa zea* as test insects.

FY94 & FY95 WORK PLANS: Extracts and volatile collections of the 10 attractive species will be analyzed for identification of individual chemical components. All identified chemicals will be obtained through commercial channels or by synthesis, and tested for biological activity in laboratory or field tests. All extracts and volatile collections will also be fractionated, and biological activity followed in various fractions.

INVESTIGATOR'S NAME(S): R. Teranishi, S. Kint, and D. M. Light

AFFILIATION & LOCATION: USDA, ARS, WRRC, Albany, CA

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.2 Develop and implement methods to suppress *Heliothis/Helicoverpa* populations with attracticide (allelochemical) baits for adults

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Because flowers of the *Gaura* species are attractive to *Heliothis/Helicoverpa*, flowers from *Gaura drummondii*, *G. suffulta*, and *G. longiflora* were picked and extracted with hexane by the Texas team, headed by P. D. Lingren. Extracts were concentrated, distilled, and analyzed by the WRRC team, headed by R. Teranishi. Twelve, twenty-one, and twenty-five compounds were identified in the respective *Gaura* species flowers. Subsequently, the *Gaura* flowers have been extracted sequentially with hexane, ethyl ether, and methanol. Volatiles in these fractions are being analyzed.

FY94 & FY95 WORK PLANS: Isolates and fractions designated as attractive will be analyzed in detail, with emphasis on the more active materials indicated by quantitative bioassays. Synthetic mixtures of active compounds will be made for optimum attraction for use in formulating attracticides.

INVESTIGATOR'S NAME(S):		J. K. Westbrook, K. R. Beerwinkle, P. D. Lingren, and T. N. Shaver
AFFILIATION & LOCATION:		USDA, ARS, CIPMRU, College Station, TX
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.2	Develop and implement methods to suppress *Heliothis/Helicoverpa* populations with attracticide (allelochemical) baits for adults
OPTIM ARRAY:	4.2.2a	Select effective chemical toxicant and formulation for adults that do not interfere with the efficiency of the attractant
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: An automated weather station has been deployed in the center of a 20m x 20m plot of sweet corn. The weather station is instrumented with anemometers and wind vanes at several heights from 0.43m to 3.20m above ground level to measure the vertical profile of wind velocity within the developing corn canopy. Further, a three-dimensional propeller anemometer and three hot film anemometers are included among the sensors to measure high-frequency turbulence. The weather station in the center of the field and one at 2m outside the prevailing upwind perimeter of the plot are instrumented with air temperature, soil temperature, relative humidity and soil moisture sensors. Atmospheric dispersion values will be calculated to determine the amount of lateral and vertical mixing that could disperse plant derived allelochemicals above and under the corn canopy.

FY94 & FY95 WORK PLANS: Automated weather stations will be deployed at various heights in sweet corn and other crops to establish lateral gradients of the atmospheric wind flow and dispersion. Ion generators and detectors will be placed in the fields to establish the spatial dispersal and distribution of neutrally-buoyant substances for quantifying and modeling the dispersal of allelochemicals within the plant canopy. Mean dispersal rates of various plant derived volatiles will be measured by vacuum sampling of air through an adsorbing substrate for several hours at locations in the field plots and analyzing the concentration of the allelochemicals by gas chromatography/mass spectrometry. Vaporization rates of various allelochemicals from several substrates used as lures will be measured in environmental chambers under controlled temperature, relative humidity and wind speed conditions. Insect behavioral response to dispersing allelochemicals in the field plots will be observed using night vision equipment.

INVESTIGATOR'S NAME(S):		D. E. Hendricks
AFFILIATION & LOCATION:		USDA, ARS, SFCIML, Stoneville, MS
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.3	Develop and implement methods to use pheromone mating disruption as an economically effective and reliable strategy for managing *Heliothis/Helicoverpa* species
OPTIM ARRAY:	4.3.2	Develop mechanized methods compatible with grower operations for distributing pheromone formulations
DATES COVERED BY REPORT:		December 1992-August 1993

PROGRESS REPORT: Two 35-acre research plots were established in the mid-Delta region of the Miss. river. A system of deploying a known dosage of pheromone chemicals was developed that will assure their dispersal within the canopy of cotton plants cultivated in one of the research plots. Infestation rates of bollworm or tobacco budworm eggs or larvae as determined by inspecting plants in both treated and untreated plots will be compared. Captures of moths in pheromone traps installed in both treated and untreated plots will also be compared along with yield of cotton from each plot. Prior to blooming of the cotton, moth captures and infestation rates in the field where pheromone was deployed were less than those in the untreated field. Pheromone is being deployed during the period between the appearance of the first bud (square) and the first open boll (fruit). For disruption of the mating process, initial deployment of pheromone was on June 18, 1993 in these field plots.

FY94 & FY95 WORK PLANS: Systems of dispensing controlled dosages of pheromone chemicals will be developed and tested in field conditions, and effects of pheromones on insect behavior and infestation rates in cultivated host crops will be studied.

INVESTIGATOR'S NAME(S):		M. S. Mayer
AFFILIATION & LOCATION:		USDA, ARS, IABBBRL, Gainesville, FL
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.3	Develop and implement methods to use pheromone mating disruption as an economically effective and reliable strategy for managing *Heliothis/Helicoverpa* species
SUPPL ARRAY:	4.3.3	Evaluate selected nonpheromone chemicals as potential mating disruptants
DATES COVERED BY REPORT:		September 1992-June 1993

PROGRESS REPORT: Two synthetic pheromone analogues synthesized by Dr. R. E. Doolittle were tested to determine whether or not they would synergize the response of the antennal olfactory neuron of *H. zea* that is specialized to detect (Z)-7-hexadecenal. Although both of the two synergens were acetates, recordings from seven individual olfactory neurons showed that one of the two analogues synergized the response.

FY94 & FY95 WORK PLANS: Both electrophysiological and behavioral assessments of synergism with newly synthesized aldehyde analogues will be begun to determine whether or not they will synergize the response of the receptor neuron in addition to the behavioral response. If synergism of (Z)-7-hexadecenal is effected it means that the analogue is effective at the active receptor site. The synergen has an attachment site that can be made more chemically reactive. It is hoped that other more reactive analogues will covalently bind within this site. If this happens it is expected that the moth will be rendered unable to detect more of the main pheromone component. Active analogues will be assayed both behaviorally and electrophysiologically.

INVESTIGATOR'S NAME(S): E. R. Mitchell, J. R. McLaughlin, and F. C. Tingle

AFFILIATION & LOCATION: USDA, ARS, IABBBRL, Gainesville, FL

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.3 Develop and implement methods to use pheromone mating disruption as an economically effective and reliable strategy for managing *Heliothis/Helicoverpa* species

DATES COVERED BY REPORT: June 1991-October 1992

PROGRESS REPORT: Small plot field trials were conducted in tobacco and/or cotton in 1991 and 1992 to evaluate different pheromone components, pheromone blends and formulations as mating disruptants for *Heliothis virescens* and *Helicoverpa zea*. Formulated materials were provided by Hercon Environmental Company, Emigisville, PA, Pherotech Inc., Delta, B. C., Canada, and Shin-Etsu, Tokyo, Japan.

The test materials were evaluated in 60 ft^2 plots of tobacco (early season) or cotton. The dispensers were attached to survey flags placed in the row or directly on plants. The effectiveness of the treatments over time was measured by (1) 'shutdown' of moth captures in pheromone-baited wire-cone traps and (2) reductions in mating of sentinel females (8-10) placed on mating tables positioned near the center of the treated plots versus untreated controls.

Of the various formulations evaluated, only the Shin-Etsu 'twist tie' rope has provided near 100% mating disruption for both species for more than a few days. In the 1991 trials, the 'twist ties' reduced mating by *H. virescens* and *H. zea* for 5-6 weeks in tobacco, but only 3 weeks in cotton. The primary difference between the tests was that the trials in tobacco were conducted in late spring and early summer when ambient temperatures were somewhat cooler.

Shin-Etsu changed the formulation for the 1992 trials and also increased the quantity of A.I. from ca. 90 mg to 165 mg total pheromone/dispenser. Various combinations of two- and three-component pheromone blends were evaluated in cotton at dosages of ca. 775 and 2,325 dispensers/acre.

The most effective pheromone treatment for both Hv and Hz was a blend of Z-11-hexadecenal/Z-9-tetradecenal/Z-9-hexadecenal in a ratio of 15:1:1. Z-ll-hexadecenal is the major component of the pheromone blend for both Hv and Hz; Z-9-hexadecenal and Z-9-tetradecenal are minor but essential components of Hv and Hz pheromone systems, respectively. The 3-component blend gave near 100% mating control for >70 days.

FY94 & FY95 WORK PLANS: A Pilot Program "Control of *Heliothis/Helicoverpa* Complex and Armyworm in Cotton with Semiochemicals" was initiated in FY 93. Small plot trials of the 3-component pheromone blend provided by Hercon, Pherotech, Shin-Etsu, and Scentry will be evaluated to establish the most efficacious formulation in terms of longevity and dosage (units/acre). The most effective formulation selected from the 1993 trials will be applied to 125-250 acres of cotton in 1994 as a component of an integrated control program for lepidopterous pests.

INVESTIGATOR'S NAME(S):		K. R. Beerwinkle
AFFILIATION & LOCATION:		USDA, ARS, CIPMRU, College Station, TX
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.4	Develop and implement methods for using siochemicals to improve estimates of *Heliothis/Helicoverpa* populations and detect exotic species
SAFEGD ARRAY:	4.4.1:	Determine attractiveness of allelochemicals to other economic lepidopterous pests
OPTIM ARRAY:	4.4.2	Develop effective trapping systems
DATES COVERED BY REPORT:		September 1991-July 1993

PROGRESS REPORT: No research conducted in these areas during the reporting period.

FY94 & FY95 WORK PLANS: Research will be conducted on trapping systems using semiochemicals as baits as effective synthetic semiochemicals are developed.

INVESTIGATOR'S NAME(S): D. E. Hendricks

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.4 Develop and implement methods for using semiochemicals to improve estimates of *Heliothis/Helicoverpa* populations and detect exotic species

OPTIM ARRAY: 4.4.2 Develop effective trapping systems

DATES COVERED BY REPORT: January 1990-August 1993

PROGRESS REPORT: Numbers of moths captured in single pheromone traps set at least 150 meters apart in different environmental conditions were compared with average numbers of moths caught in clusters of 3 to 5 traps where traps were installed about 50 ft apart in conditions similar to those where single traps were installed. Results show that day-to-day extremes were moderated when numbers of moths caught in clusters of traps were averaged and accepted as daily counts. Captures in single traps, installed 150 m or more apart, indicated that vegetative, geographic, and environmental conditions at specific trap sites can significantly influence performance of each single trap, and day-to-day variations were more extreme and erratic.

Field experiments were completed to determine the accuracy of an automatic system to detect bollworm and tobacco budworm moths attracted to baits made of species-specific sex pheromone blends. The system included a telemetry system that sent a radio signal to a receiver and computer when a moth was detected at night. The computer was programmed to collate the number of moths detected from each detector unit installed in the field near host crops. Overall accuracy throughout a 6-month growing season was >98%, and accuracy ranged from 87% when numbers of moths detected or caught per night were high (>25) to 100% when numbers caught or counted per night were low (0 to 24). No significant differences were found between numbers of either of these species caught in traps equipped with detectors or in traps without detectors.

FY94 & FY95 WORK PLANS: Year-round surveys for bollworm and tobacco budworms moths using clusters of traps baited with pheromone will continue in the mid-Delta region of Miss. Annual population profiles will be derived from these data, compared with climatic conditions, and incorporated in computerized population prediction models.

INVESTIGATOR'S NAME(S):		J. D. Lopez, Jr.[a], M. A. Latheef[b] & J. A. Witz[a]
AFFILIATION & LOCATION:		[a]USDA, ARS, CIPMRU, College Station, TX; [b]USDA, ARS, AARU, College Station, TX; [c]Formerly USDA, ARS, PMRU, College Station, Currently, Texas Transportation Institute, Texas A&M University, College Station, TX
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.4	Develop and implement methods for using semiochemicals to improve estimates to estimates of *Heliothis/Helicoverpa* populations and detect exotic species
OPTIM ARRAY:	4.4.2	Develop effective trapping systems
DATES COVERED BY REPORT:		September 1991-July 1993

PROGRESS REPORT: Analyses of data collected during the Pilot Test titled "Area-wide Management of *H. zea* and *H. virescens* by Pheromone Trap Calibration" which was conducted from 1987-89 are continuing. Analyses of data on captures of corn earworm in pheromone traps and during nocturnal sampling and the relationship to egg and adult densities in field corn indicated that: (1) the percentage of nightly pheromone trap catch was highest between 2100 and 2200 h (CDT), (2) coefficient of determination between number of males caught in traps between 2100 and 2200 h and egg densities determined the following morning ($R^2=37\%$) was also higher than for other periods, (3) stepwise regression showed that trap catch between 0400 and 0500 h in combination with numbers of fresh silks per ha provided the best equation for predicting egg densities ($R^2=51\%$), (4) 62% of all adults captured during nocturnal sampling were females with the highest percentage being caught between 2100 and 2400 h; male captures were nearly uniform throughout the night, (5) percentage of mating pairs was highest between 0300 and 0400 h, (6) temporal patterns of captures of males in the pheromone traps and mating pairs did not reflect competition between the traps and native females; there was no decrease in the fraction of males captured in pheromone traps when mating activity was highest and (7) there were significant linear regression relationships between the number of corn earworm male moths caught per pheromone trap per night and density of females ($R^2=67\%$) and males ($R^2=69\%$) estimated from nocturnal sampling. An M.S. graduate student in Statistics is currently evaluating the relationship between captures of males in pheromone traps and density estimates from nocturnal sampling for corn earworm in cotton. This analysis is similar to that reported by Witz et al. (1992) for the tobacco budworm on cotton. Two different sizes of malaise traps (1 m and 6 m) were evaluated for capturing corn earworm, tobacco budworm and a number of other noctuids in an area containing dense stands of ergot-infected dallisgrass. Both trap sizes were effective in capturing corn earworm, tobacco budworm, and other noctuids including black cutworm, beet armyworm, true armyworm, green cloverworm, cotton leafworm, variegated cutworm, fall armyworm, cabbage looper, soybean looper, and others. The efficiency of the two sizes of malaise traps was not proportional to the area indicating that other factors such as the height of the traps may influence capture. In addition to monitoring adult activity of noctuids, the malaise traps have the potential to be used to bioassay the attractiveness of plants or chemicals.

FY94 & FY95 WORK PLANS: Analyses of pilot test data will be continued. Evaluations of malaise traps for monitoring the activity of corn earworm and other noctuids by trapping in areas containing plants that have been shown to attract noctuids will be continued. Malaise traps will be used to evaluate the response of corn earworm and other noctuids to different semiochemical concentrations represented by different numbers of attractive sources such as plants or chemical dispensers. Malaise traps of different heights will be evaluated to determine if efficiency can be increased.

INVESTIGATOR'S NAME(S): A. K. Raina

AFFILIATION & LOCATION: USDA, ARS, INHRL, Beltsville, MD

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.4 Develop and implement methods for using semiochemicals to improve estimates of *Heliothis/Helicoverpa* populations and detect exotic species

DATES COVERED BY REPORT: October 1991-August 1993

PROGRESS REPORT: In collaboration with Dr. Gabor Szocs of Hungary, identified and field tested the pheromone of *Heliothis maritima*, found in Eastern Europe. In addition we have done the preliminary identification of the sex pheromone of *Heliothis viriplaca*, also collected in Eastern Europe.

FY94 & FY95 WORK PLANS: Various combinations of the *Heliothis viriplaca* pheromone blend will be tested in the flight tunnel and in the fields in Hungary.

INVESTIGATOR'S NAME(S):		A. K. Raina
AFFILIATION & LOCATION:		USDA, ARS, INHRL, Beltsville, MD
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.5	Develop methods to interfere with neuro-endocrine control of pheromone biosynthesis in *Heliothis/ Helicoverpa* species
OPTIM ARRAY:	4.5.2	Develop practical means to apply neuropeptides, biogenic amines and other identified substances to insects to interfere with or manipulate pheromone biosynthesis
SUPPL ARRAY:	4.5.3	Investigate factors in the environment including plant produced substances that may educt the regulating of pheromone production via interfering with or inducing the action of neuropeptides and other endogenous factors.
DATES COVERED BY REPORT:		October 1991-August 1993

PROGRESS REPORT: An extensive study of the structure activity of a pheromone biosynthesis activating neuropeptide (PBAN), revealed that the C-terminal pentapeptide is the minimum biologically active sequence, and that a pentapeptide ($PBAN_{15-19}$) is a super agonist. Generated a polyclonal antibody to PBAN, and developed specific ELISA and RIA. Using immunohistochemistry, mapped the cells in the suboesophageal ganglion that produce PBAN. Cloned and sequenced the gene for Hez-PBAN. Cloned the PBAN gene into a baculovirus and obtained *in vitro* expression. A 110 kD carrier protein for PBAN has been detected. Work is in progress to identify the receptor for PBAN. A pheromonostatic peptide (PSP) was isolated from the accessory glands of male *H. zea*, and it's amino acid sequence (57 residues) determined. PSP is transferred by the male to the female at the time of mating and terminates pheromone production.

Designed and synthesized a pseudo-mimic of PBAN with one amino acid and an organic molecule having pheromonotropic activity. Females of *H. zea* infected with AcNVP into which PBAN gene was cloned did not produce pheromone during photophase as expected. Demonstrated that PBAN and some of its analogs can cause pheromonotropic response when fed to photophase females.

Females of *H. zea* and *Heliothis virescens* reared from field collected larvae and their F_1 and F_2 generations did not produce pheromone unless exposed to a host plant (corn, cotton, tobacco). Several corn silk volatiles, including the host plant hormone ethylene, were able to induce pheromone production in such females. Preliminary experiment indicate that these host factors induce pheromone production by causing the release of PBAN.

FY94 & FY95 WORK PLANS: Continue research towards the identification of PBAN receptor. Synthesize PSP, determine the minimum biologically active sequence and it's mode of action. Continue design of antagonistic PBAN mimics and obtain a specific vector for engineering PSP gene. Investigate the effect of other volatile components from cotton and tobacco in *H. virescens*. Confirm their mode of action. Determine how rearing in the laboratory eliminates the requirement of host plants for pheromone production.

INVESTIGATOR'S NAME(S): P.E.A. Teal[a], J.H. Tumlinson[a], R.L. Abernathy[a], T.C. Christensen[a], J.G. Hildebrand[a], N.T. Davis[b], R.J. Nachman[b] and G.M. Holman[c]

AFFILIATION & LOCATION: [a]USDA, ARS, IABBBRL, Gainesville, FL; [b]Division of Neurobiology, University of Arizona, Tucson, AZ; [c]USDA, ARS, FAPRL, College Station, TX

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.5 Develop methods to interfere with neuro-endocrine control of pheromone biosynthesis in *Heliothis/Helicoverpa* species

SAFEGD ARRAY: 4.5.1 Screen compounds including biogenic amines and other peptides that have been identified from insects and shown to have physiological activity for their effects on pheromone biosynthesis

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS: Studied the role of the central nervous system in regulating sex pheromone production in *Heliothis virescens*. Showed that neurosecretory cells containing pheromone biosynthesis activating neuropeptide (PBAN) extend to the retrocerebral complex as well as down the ventral nerve cord to the terminal abdominal ganglion (TAG). The TAG contained pheromonotropic peptides in sufficient quantities to stimulate sex pheromone production. Three different pheromonotropic peptides were present in the TAG and none of them have the same sequence as PBAN of *Helicoverpa zea*. The three peptides have the same retention characteristics on a variety of HPLC columns as PBANs isolated from the brain-subesophageal ganglion (BR-SEG) of *H. virescens*.

The C-terminal five amino acids of PBAN share sequence homology with pyrokinin neuropeptides (phe-X-pro-arg-leu) (X=thr,val,gly or ser). Studied 12 naturally occurring pyrokinins or fragments and found relationships between PBAN and the target organ. The fourth amino acid from the C-terminus is critical for activity and pyroglutamate protection of the amine terminus can have a superagonistic effect. Discovered the C-terminal five amino acids are required for activity and activity is increased by addition of other amino acids to this fragment.

Pheromone production by virgin females of *H. zea* decreased with age and was inversely correlated with the deposition of unfertilized eggs. Discovered a factor in the bursa copulatrix, ovaries and hemolymph of senescing virgin females that inhibits the action of PBAN and production of sex pheromone. Dose response studies indicate a linear decrease in sex pheromone production suggesting the material either competes with PBAN at the receptor level or combines with PBAN to form an inactive complex.

Shown that females of *H. virescens* can be induced to produce sex pheromone by injection of octopamine, and that isolated abdomens can be stimulated to produce pheromone only when treated at the onset of scotophase. Radioenzymatic assays showed that the octopamine level in the TAG was high during the photophase and declined at the onset of photophase; levels in the pheromone gland were low during photophase but increased at the onset of scotophase.

FY94 & FY95 WORK PLANS: Isolate and identify pheromonotropic peptides present in the BR-SEG and TAG and determine other physiological effects of these peptides. Determine the role of octopamine and other biogenic amines play in regulating pheromone production. The factor responsible for inhibition of sex pheromone production will be identified. Structure activity studies will be conducted to identify the mode of action of this factor. Stable synthetic analogues that are protected from degradation will be synthesized and tested for bioactivity facilitate the development of a delivery system this factor.

INVESTIGATOR'S NAME(S):		P. E. A. Teal
AFFILIATION & LOCATION:		USDA, ARS, IABBBRL, Gainesville, FL
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.6	Develop methods to disrupt the enzymatic systems in the pheromone biosynthetic pathway to suppress pheromone production
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Results of studies in which females of *H. virescens* and *H. subflexa* were artificially stimulated to produce pheromone by injection of pheromone biosynthesis activating neuropeptide have shown a direct correlation between the amount of free (Z)-11-hexadecenoic acid present in the pheromone gland and the amount of the major pheromone component of both species, (Z)-11-hexadecenal. No such correlation exists for the bound fatty acid. Deuterium labelling studies have shown that topically applied 16,16,16-D_3 hexadecanoic acid is converted to (Z)-11-hexadecenal by pheromone glands of both species and that biosynthesis of the aldehyde involves the action of acetate esterases and alcohol oxidases as demonstrated previously. Studies conducted to assess the rates at which species specific blends of pheromone components are established after artificial induction of pheromone biosynthesis have shown that blends are established within the first 10 min after induction of pheromone biosynthesis. During the first 10 min amounts of acetate and alcohol precursors are higher than those found in sexually attractive females. Studies conducted using interspecific hybrids between the two species have shown that autosomal genes regulate the expression of esterase and oxidase activities as well as enzymatic steps involved in production of tetradecanal and (Z)-9-tetradecenal. Results of hybridization experiments have also shown that the *H. virescens* genome exerts primary control over establishment of pheromone blends by female hybrid and backcross insects.

FY94 & FY95 WORK PLANS: The effects of pheromonotropic neuropeptides on enzyme induction will be explored as will the effects of recently isolated antagonists to pheromone production.

INVESTIGATOR'S NAME(S): D. M. Jackson

AFFILIATION & LOCATION: USDA, ARS, CRL, Oxford, NC

ACTION AREA: 4. Behavior-Modifying Chemicals

LEAD ARRAY: 4.7 Develop and implement strategies for using semiochemical- enhanced parasitiods to achieve economical, effective and reliable biological control of *Heliothis/Helicoverpa*

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Over the past six years (1988-1993) adult *Cardiochiles nigriceps* wasps, a parasitoid of tobacco budworm larvae, have been counted as they flew over replicated three-row plots of 117 accessions of 67 *Nicotiana* species (including tobacco, *N. tabacum*). *C. nigriceps* is attracted to several *Nicotiana* species in the absence of their insect hosts. *Nicotiana noctiflora* and *N. sanderae* were especially attractive to *C. nigriceps*. Periodically over the last three years, wasps were netted as they flew over flowering tobacco, two accessions of *Nicotiana noctiflora*, and three accessions of *N. sanderae*. About 90% of the wasps flying over tobacco were females, while only about 25% of the wasps captured over *N. noctiflora* were females. Equal numbers of *C. nigriceps* were observed flying over flowering and non flowering (topped) *N. noctiflora* plants. Large quantities of leaf material from *Nicotiana* species that were most attractive to *C. nigriceps* were extracted with methylene chloride and frozen immediately on dry ice.

FY94 & FY95 WORK PLANS: *Nicotiana* species extracts are being used for the isolation of volatile components for bioassays against *C. nigriceps*. This is in cooperation with Drs. W. Schlotzhauer and R. F. Severson (USDA-ARS, Athens).

INVESTIGATOR'S NAME(S):		J. H. Tumlinson and W. J. Lewis
AFFILIATION & LOCATION:		USDA, ARS, IABBBRL, Gainesville, FL
ACTION AREA:	4.	Behavior-Modifying Chemicals
LEAD ARRAY:	4.7	Develop and implement strategies for using semiochemical-enhanced parasitoids to achieve economical, effective and reliable biological control of *Heliothis/Helicoverpa*
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: When *Heliothis/Helicoverpa* larvae feed on corn, cotton, soybean, or cowpea plants the plants actively produce/release volatile compounds. These compounds attract the parasitoid females of the species *Microplitis croceipes* and *Cotesia marginiventris* and thus aid them in finding their larval hosts. There are great differences in the volatile blends produced by different species of plants and thus the wasps need to be able to learn in order to find their polyphagous hosts in a wide variety of chemical environments. The response by the plant is caused by a substance in the oral secretions of the larvae and the plants do not respond to artificial damage in the same way they respond to larval damage. Furthermore, the plant response is systemic and damage of one leaf by a larva will cause the entire plant to emit volatile chemical signals. Also, different varieties of a plant species respond differently to larval damage. A wild variety of cotton produces about ten times the amount of volatiles as domestic varieties we have studied, when fed on by caterpillars. Some domestic varieties of cotton and corn produce very little volatile chemical signal in response to larval damage. Manifestly, this information should be useful in the development of resistant varieties of various crops. The results we have thus far and those of our colleagues in the Netherlands indicate that this phenomenon may be very general and part of the defense mechanisms used by plants.

FY94 & FY95 WORK PLANS: Identify factor in larval oral secretions that induces plants to emit chemical signals that attract parasitoids. Determine mechanism of action of this substance. Investigate several varieties of cotton and other plants to determine whether some varieties are more attractive to natural enemies of *Heliothis/Helicoverpa* than other varieties and whether an attractive variety can be developed through breeding or gene transfer. Study the action of the active factor(s) from larval oral secretions on several species of crops and cover crops to determine which are likely to be most attractive to parasitoids and thus most likely to provide refuge for natural enemies. Investigate plant chemicals associated with location of food resources (e.g. extrafloral nectaries for parasitoid wasps).

TABLE 4. Summary of Research Progress for Action Area IV, Behavior Modifying Chemicals, in Relation to Year 2 Goals of the 5-Year Plan.

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
4.1 Develop and implement methods to manage *Heliothis/Helicoverpa* populations in cropping systems with plant derived allelochemicals.	Isolate and identify oviposition attractants, stimulants, and deterrents and conduct laboratory and field cage bioassays.	X		Potentially useful sources of attractants, feeding and oviposition stimulants have been discovered from extracts of cotton, tobacco, several species of *Gaura* and *Desmodium tortuosum*. The flowers and fruiting forms seem to be the source of the most active extracts and volatile substances. An ion generator system is being developed for measuring air mixing in corn fields. Crude extracts of a mint species caused some deterrence to oviposition by corn earworm on corn silks however neem oil was not an effective deterrent.
4.2. Develop and implement methods to suppress *Heliothis/Helicoverpa* populations with attracticide (allelochemical) baits for adults.	Isolate plant kairmones that attract adults to feed and begin identification of active chemicals.	X		Emphasis in this array has been the isolation and identification of plant volatiles from several *Gaura* species that attract adults of *Heliothis/Helicoverpa*. Volatiles from *Gaura* spp. flowers enhanced capture of several lepidoptera including *Heliothis/ Helicoverpa*. Several active isolates have been obtained from these plants and from the flowers of a species of vine and from orange and grapefruit blossoms. These isolates are undergiong extensive qualitative analysis supported by behavioral bioassays. Other research showed that commercial phagostimulants were not effective adult feeding stimulants for delivery of toxicants. Sugars, particularly sucrose appeared to be promising phagostimulants.
4.3 Develop and implement methods to use pheromone mating disruption as an economically effective and reliable strategy for managing Heliothis/Helicoverpa.	Establish research plots and begin evaluating various pheromone blends and formulations.	X		A 3-component blend of (Z)-11-hexadecenal, (Z)-9-hexadecenal, and (Z)-9-tetradecenal was the most efficacious blend for simultaneous mating disruption of *H. virescens* and *H. zea*. Dispensed from the Shin-Etsu 'twist-tie', this blend resulted in near 100% mating disruption on mating tables. Two synthetic pheromone analogs synergized the response of olfactory neurons of *H. zea* to the primary sex pheromone chemical.

TABLE 4. - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
4.4 Develop and impelement methods for using semiochemicals to improve estimates of *Heliothis/Helicoverpa* populations and detect exotic species.	Evaluate blends and formulations of sex attractant pheromones and other chemical attractants for trapping efficacy of males and females in field settings.	X		Emphasis has been on the development of methods to interpret pheromone trap captures. Population monitoring with non-pheromone semiochemicals requires identification and synthesis of active materials. Analyses of data from the Pheromone Trap Calibration Pilot Test has indicated relationships between trap capture and oviposition by corn earworm. Contrary to previously published information, these data have also indicated that competition with wild females did not affect the magnitude of trap captures. Malaise traps were evaluated and appear promising for measuring activity of noctuid species. Averaging capture of males from clusters of traps moderated the day-to-day extremes observed from widely separated traps. The sex pheromones of two Eastern European species, *H. maritima* and *H. viriplaca* have been identified. No progress was made in determining if plant kairmones can be used to habituate *Heliothis/Helicoverpa.*
4.5 Develop methods to interfere with neuro-endocrine control of pheromone biosynthesis in *Heliothis/Helicoverpa.*	Elucidate mechanism of action and receptors for factors regulating pheromone biosynthesis; conduct structure activity studies.	X		Much of the research in this area related to the structure and activity of PBAN. The minimum biologically active sequence was determined and the cells in the suboesophageal ganglion producing PBAN were mapped. The PBAN gene has been cloned and expressed *in vitro.* Pheromone production did not occur in *H. zea* females infected with AcNPV carrying the PBAN gene. Males of *H. zea* were feound to transfer a pheromonostatic peptide at the time of mating that terminates pehromone production. Females of *H. zea* and *H. virescens* reared from field collected larvae and thier F_1 and F_2 generations did not produce pheromne unless exposed to a host plant. Research in this area is very active.

TABLE 4. - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
4.6 Develop methods to disrupt the enzymatic systems in the pheromone biosynthetic pathwas to suppress pheromone production.	Isolate/characterize enzymes contolling biosynthesis and begin development of antagonists for enzyme systems.	X		Research to date has focused on determining the biosynthetic processes in *H. virescens* and *H. subflexa.* No activity is reported for 4.6.1 or 4.6.3.
4.7 Develop and implement strategies for using semiochemical-enhanced parasitoids to achieve economical, effective and reliable biological control of *Heliothis/Helicoverpa.*	Devlop methods to apply semiochemicals for conditioning parasitoids before release; develop effective semiochemical formulations for use in field tests; develop small-scale field test evaluation methods.	X		Salivary secretions from *Heliothis/Helicoverpa* larvae feeding on corn, cotton, soybean, or cowpea cause plants to release species specific volatile compounds attractive to *Micorpletis croceipes* and *Cotesia marginiventris*. A prototype semiochemical-baited trap was tested for M. croceipes. *Nicotiana noctiflora* and *N. sanderae* were found to attract *Cardiochiles nigriceps*. Isolation and identification of volatile components from these plants is underway. Major emphasis in this array was on the volatile cues produced by lepidopterous larvae feeding on plants, chemical responses of plants to injury, and the learned responses of parasitoids that respond to these chemical cues.

RESEARCH SUMMARY: ACTION AREA IV—BEHAVIOR-MODIFYING CHEMICALS

Compiled by J. McLaughlin and A. K. Raina

The research in lead arrays 4.1 and 4.2 is largely overlapping and future plans and objectives in these areas should be consolidated.

LEAD ARRAY 4.1: Research in this array is proceeding as planned with the exception of SUPPL 4.1.3 (plant radiation) in which no activity was reported. Research emphasis is greatest in the area of potential attractants, feeding stimulants, and oviposition stimulants. Continued progress may be hampered by a lack of identified active compounds. Research activity is reported from Oxford, NC; College Station, TX, Lane, OK; Albany, CA; Gainesville, FL; and Stoneville, MS.

Potentially useful sources of attractants, feeding stimulants, and oviposition stimulants have been discovered in volatiles from extracts of cotton, tobacco, several species of *Gaura* and *Desmodium tortuosum*. *Albutilon theophrastis* (velvetleaf) is a favored wild host of both species and a potential source of similar allelochemicals. The flowering and fruiting parts of these plants seem to be the source of the most active extracts and volatile substances.

A system using ion generators for measuring the lateral and vertical mixing of air that could disperse plant allelochemicals is being developed within corn fields. This system would have application to research throughout Area 4.

Crude extracts of a mint species caused limited deterrence to oviposition by corn earworm on corn silks; neem oil was not an effective deterrent.

Volatiles from flowers of certain *Gaura* species will synergistically enhance the capture of several lepidopteran species, including *Heliothis/Helicoverpa*, in pheromone-baited traps.

Nicotiana kawakamii was not clearly effective as a trap crop for protecting flue-cured tobacco from infestations of tobacco budworm and tobacco hornworm over two seasons.

LEAD ARRAY 4.2: Research in this array is primarily on the isolation and identification of bioactive (attraction, oviposition, or feeding) agents from plant flowers and applies also to the plans for Lead Array 4.1. Research is proceeding as planned with the exception of OPTIM 4.2.2b, protocols for testing phytoattractants, and SUPPL 4.2.3, evaluation of attractancy of non-plant synthetic chemicals, for which no progress or plans were reported. Future progress will largely depend upon the identification and synthesis of the active plant compounds thus far isolated. Research activity is reported from Albany, CA; Lane, OK; and College Station,TX.

Emphasis is on the isolation and identification of plant volatiles from flowers of several *Gaura* species that attract adults of *Heliothis/Helicoverpa*. Several active isolates have been obtained from these plants and from the flowers of a species of vine and from orange and grapefruit blossoms. These isolates are undergoing extensive qualitative analysis supported by behavioral bioassays.

Volatiles from flowers of certain *Gaura* species will synergistically enhance the capture of several lepidopteran species, including *Heliothis/Helicoverpa*, in pheromone-baited traps.

Commercial phagostimulants were not effective feeding stimulants for the delivery of toxicants to adult moths. Sugars, particularly sucrose, appeared promising. Variable responses from field-collected adults indicated that further studies should be conducted to identify the optimum sugars and their concentrations as well as environmental and physiological factors affecting the feeding response of the adults.

LEAD ARRAY 4.3: Research is proceeding as planned. Research in SAFEGD 4.3.1 is not scheduled to begin until year 3. Research progress is reported from Stoneville, MS and Gainesville, FL.

A 3-component blend of the primary pheromone component, (Z)-11-hexadecenal, with (Z)-9-hexadecenal and (Z)-9-tetradecenal was determined to be the most efficacious for simultaneous disruption of mating of *Heliothis virescens/Helicoverpa zea*. This blend in the Shin-Etsu 'twist-tie' formulation gave near 100% mating disruption, as measured with mating tables, for >70 days. New dispensing technology currently is being investigated for dispersal of the pheromone throughout the plant canopy.
Two synthetic pheromone analogs synergized the response of olfactory neurons of *Helicoverpa zea* to the primary sex pheromone chemical.

FY 94 & 95 WORK PLANS: A Pilot Program "Control of *Heliothis/Helicoverpa* Complex and Armyworms in Cotton with Semiochemicals" was initiated in FY 93. Small plot trials of the 3-component blend will be evaluated in various commercial formulations to establish the most efficacious. This formulation will be applied to 125-250 acres of cotton in 1994 as a component of an integrated control program for lepidopterous pests. Further development of systems for dispensing pheromones within the plant canopy will be developed and evaluated using criteria of trap shutdown, reduction of mating, and infestation and damage levels. New pheromone analogs will be synthesized and evaluated electrophysiologically and behaviorally for chemical reactivity with the pheromone binding site in order to develop analogues that will render the male moth incapable of detecting the female pheromone. Research will be initiated in 4.3.1 if suitable pheromone-treated areas are available for study.

LEAD ARRAY 4.4: Emphasis in the array is presently on development of methodologies for interpretation of pheromone trap captures. No activity is reported for SUPPL 4.4.3, Determine if plant kairomone can be used to habituate *Heliothis/Helicoverpa* species. Monitoring of populations with semiochemicals other than pheromones will not progress until identification and synthesis of active materials is accomplished. Activity is reported from Beltsville, MD; Stoneville, MS; and College Station TX.

Analysis of data obtained during the 1987-1989 Pilot Test "Area-Wide Management of *H. zea* and *H. virescens* by Pheromone Trap Calibration" is progressing. Indicators that equate pheromone trap captures of corn earworm in corn with egg densities and density of females were developed. Competition with wild females was not a factor in the magnitude of trap captures. Analysis of trap data from cotton is underway.
Malaise traps were evaluated and appear promising for measuring the activity of noctuid species in relation to host plants.

Averaging the captures of males from clusters of pheromone-baited traps moderated the day-to-day extremes in the data from traps deployed in cotton. Vegetative, geographic, and environmental conditions at specific trap sites influenced the performance of widely-spaced individual traps, resulting in more variable and erratic trapping data.

The sex pheromone of *Heliothis maritima* was identified and a preliminary determination of the sex pheromone of *H. viriplaca* was made. These are both Eastern European species.

LEAD ARRAY 4.5: This is a very active area of research with reports from ARS locations at Beltsville, MD; Gainesville, FL; and College Station, TX. Cooperative support is supplied by the University of Arizona, Tucson. Research extends from the initial identification of a pheromone biosynthesis activating neuropeptide from *Helicoverpa zea* (Hez-PBAN).

Progress has occurred on the structure and activity of PBAN as well as the detection of a carrier protein.. The minimum biologically active sequence and a pentatpeptide that is a super agonist have been determined. Immunochemical techniques were developed and used to map the cells in the suboesophageal ganglion that produce PBAN. The gene for Hez-PBAN was cloned and sequenced. The PBAN gene was cloned into a baculovirus and expressed *in vitro*. Pheromonotropic activity did not occur in females of *H. zea* infected with AcNPV into which the gene for PBAN had been cloned; however a response was obtained when PBAN and

some analogs were fed to females. A pseudo-mimic of PBAN with one amino acid and an organic molecule exhibited pheromonotropic activity.

Neurosecretory cells in *H. virescens* containing PBAN extend to the retro cerebral complex and down the ventral nerve cord terminating at the terminal abdominal ganglion (TAG). Three pheromonotropic peptides in the TAG of this species have different sequences than Hez-PBAN, but appear to be similar to PBANs isolated from the brain-suboesophageal ganglion of *H. virescens*.

Males of *H. zea* transfer a pheromonostatic peptide (PSP) at the time of mating that terminates pheromone production. Senescing virgin females of *H. zea* produce an endogenous factor in the bursa copulatrix, ovaries and hemolymph that inhibits pheromonotropic activity.

Injection of octopamine will induce pheromone production in *H. virescens* females. Octopamine levels are high in the TAG during photophase and decline during scotophase while the opposite cycle occurs within the pheromone gland.

Females of *H. zea* and *H. virescens* reared from field collected larvae and their F_1 and F_2 generations did not produce pheromone unless exposed to a host plant. Ethylene was identified as one volatile that would induce pheromone production.

LEAD ARRAY 4.6: Research to date has focused on determining the biosynthetic processes in *Heliothis virescens* and *H. subflexa*. Activity is reported from Gainesville, FL. No activity is reported for SAFEGD 4.6.1, Screen enzyme inhibitors and blockers of oxidase and esterases for compounds that may block pheromone biosynthesis. Activity relating to OPTIM 4.6.2, Develop practical methods to apply or deliver enzyme inhibitors, antagonists or agonists for control of pheromone production, is reported under ARRAY 4.5. No activity is reported for SUPPL 4.6.3, Investigate plant substances and other factors in the environment that may enhance or inhibit pheromone production through their effects on the biosynthetic pathway.

LEAD ARRAY 4.7: Major emphasis in this array is on the volatile cues produced by lepidopterous larvae feeding on plants, chemicals responses of plants to feeding injury, and the learned responses of parasitoids that respond to these chemical cues. Progress has been notable; future progress will depend upon the identification of factors responsible for plant responses and the volatile cues. Activity is reported from Gainesville, FL; Tifton, GA, and Oxford, NC.

Salivary secretions from *Heliothis/Helicoverpa* larvae feeding on corn, cotton, soybean, or cowpea cause the plants to produce/release volatile compounds that attract females of the parasitoids *Microplitis croceipes* and *Cotesia marginiventris*. The volatiles produced by different species of plants are not the same and the parasitoids must be able to learn to respond to specific odor cues in order to locate their polyphagous hosts.
Nutrition affects the performance and behavior of adult parasitoids and must be considered when attempting to enhance the abundance and effectiveness of natural or released parasitoids.

A prototype semiochemically baited trap was tested for capturing *M. croceipes*.
Nicotiana noctiflora and *N. sanderae* plants are particularly attractive to the tobacco budworm parasitoid *Cardiochiles nigriceps*. Plant material has been collected for isolation and identification of volatile components responsible for this attraction.

BREAKOUT SESSION SUMMARY

Progress reported in Action Area IV reflected a good balance between the basic and applied research on *Heliothis/Helicoverpa* group. Participants agreed that basic research relative to behavior modifying chemicals is essential for providing the vital information about a pest, and is the basis for developing control/management strategies.

The use of sex pheromones for mating disruption was considered a viable technology for use in H/H management. However, additional research to determine their efficacy in relation to host density and other ecological conditions is required to make this strategy workable for area wide management. Participants also felt that optimization of pheromone blends and dispensers is necessary before these chemicals can be used in trapping systems for estimating populations. Development of attracticide systems for suppressing adult populations was considered a viable H/H control technology. Developing such systems will require evaluating feeding attractants and other host plant factors in order to isolate, identify and formulate specific chemicals that may serve at attractants. Attracticide systems based on plant volatiles would have more potential than systems based on pheromones since both sexes would be attracted. The use of plants such as Gaura and ergot infected Dallis grass as trap crops was also considered an option in developing adult attracticide systems. Current research to identify factors such as neuropeptides that affect behavior, and studies on their mode of action may offer a new population management tool. Considerable research, including the development of satisfactory vector systems, is needed to develop methods to use these factors for altering the normal behavior of H/H. Participants considered that overall research on behavior modifying chemicals including sex pheromones, feeding attractants, oviposition stimulants and behavior modifying chemicals was progressing satisfactorily.

The group recommended combining lead Arrays 4.1 and 4.2, as well as 4.5 and 4.6, to avoid overlap and to streamline research efforts. The proposed new Lead Arrays are:

LEAD ARRAY:	4.1:	Develop and implement methods to manage *Heliothis/Helicoverpa* populations in cropping systems with plant derived allelochemicals and attracticide baits.
SAFEGD ARRAY:	4.1.1:	Determine whether plant-derived kairomone attractants enhance the efficiency of pheromone traps.
OPTIM ARRAY:	4.1.2a:	Combine plant derived kairomones with sex pheromones to monitor and/or manage both sexes.
	4.1.2c:	Develop protocols for testing phytoattractants.
SUPPL ARRAY:	4.1.3:	Develop plant derived kairomones as tools for monitoring.
LEAD ARRAY:	4.4:	Develop methods to interfere with neuro-endocrine and enzymatic systems to control pheromone production in *Heliothis/Helicoverpa* species.
SAFEGD ARRAY:	4.4.1:	Screen compounds including peptides, biogenic amines, and enzyme inhibitors and blockers of oxidases and esterases for their effect on pheromone biosynthesis.
OPTIM ARRAY:	4.4.2:	Develop practical methods to apply or deliver peptides, amines, and other active substances to insects to interfere with or manipulate pheromone biosynthesis.
SUPPL ARRAY:	4.4.3:	Investigate plant substances and other factors in the environment that may educe regulation of pheromone production.

Lead Arrays 4.3 (now 4.2) and 4.4 (now 4.3) remain unchanged.

Action Area V. BIOLOGICAL CONTROL

Coordinators: J. J. Hamm and P. G. Tillman

INVESTIGATOR'S NAME(S):		M. R. Bell
AFFILIATION & LOCATION:		USDA, ARS, SFCIPML Stoneville, MS
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.1	Development technology for managing *Heliothis/Helicoverpa* spp. using entomopathogens/nematodes
SAFEGD ARRAY:	5.1.1	Develop efficacious methods for mass producing entomopathogens/nematodes Progress
OPTIM ARRAY:	5.1.2c	Develop application technology for optimum field efficacy for particular regions of the country
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: *In vivo* production of nuclear polyhedrosis virus (NPV) utilizing *H. virescens* and *H. zea* was investigated using both reusable, multicellular rearing trays and disposable, heat-formed plastic trays. Production characteristics were examined using various concentrations of inoculum, ages of larvae, and collection dates. Three NPVs were produced: NPV from the corn earworm (*Hz*SNPV), from *H. armigera* (*Ha*MNPV), and from the celery looper (*Af*MNPV). Increases in polyhedra compared to the inoculum varied with the virus being produced and host used. Inoculating 7-day-old larvae and collecting 7 days after inoculation produced totals of ~ 5.7 billion *Ha*MNPV polyhedra/budworm larvae, 6.1 billion *Hz*SNPV polyhedra/bollworm larvae, and 1.3 billion *Af*MNPV polyhedra/budworm larvae. Small quantities (500 acre-treatments) of *Ha*MNPV and *Af*MNPV were produced for cooperative field studies. A semi-automated production system using the heat-formed trays was used to produce 22,500 acre-treatments of *Hz*SNPV at a cost considered efficacious for large area studies. This was then formulated for application by Sandoz Crop Protection, Inc. (ELCAR).

Methods of aerial application of NPV were examined to obtain increased deposition and persistence of polyhedra on early-season hosts (geranium spp.) of budworm/bollworm in the delta area of Mississippi. Although applications from heights of 15 feet produced adequate coverage (64 droplets/cm^2), when applications were made from ~ 50 feet during large area tests, only 3.3 droplets/cm^2 were deposited. The addition of 4% crop oil increased the deposition to 28 droplets/cm^2. Small aircraft treated an 18,000 acre test with virus/water/oil using onboard positioning systems for guidance. The methods used could be used for large area applications.

INVESTIGATOR'S NAME(S): T. A. Coudron

AFFILIATION & LOCATION: USDA, ARS, BCIRL Columbia, MO

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.1 Develop technology for managing *Heliothis/Helicoverpa* spp. using entomopathogens/nematodes

OPTIM ARRAY: 5.1.2a Improve efficiency through natural or genetic manipulation of entomopathogens

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: A unique toxin, capable of arresting larval-larval ecdysis in *Heliothis/Helicoverpa* spp., has been isolated from the venom of *Euplectrus comstockii* (Hymenoptera: Eulophidae) and partially characterized. The selective and potent qualities of this toxin make it an attractive candidate for enhancing the effectiveness of microbial biocides. (Accomplished during years 1-3.) The compatibility of the venom with two baculoviruses (i.e. HzSNPV and AcMNPV) has been tested as potential vector systems for the application of the toxin. In both cases the presence of the baculovirus did not alter the ability of the venom to arrest molting. At late infection stages, only minor changes were observed in the LC_{50} of HzSNPV in the presence of the venom. However, the venom significantly lowered the LC_{50} of AcMNPV. Surface response equations have been developed to visualize the effect of the venom on the virosis. (Accomplished during years 2-3.) The venom was shown to stimulate premature production of late larval storage proteins in whole animals and in isolated tissues. These findings suggest that the venom acts in a unique manner and is capable of regulating gene expression by mechanisms separate from inherent developmental processes and the intact endocrine system. (Accomplished during year 3.)

FY94 & FY95 WORK PLANS: Funding from the USDA-CSRS NRICGP has been received to complete the characterization of the toxin from the venom of *E. comstockii*, and to engineer the gene for the toxin into an AcMNPV expression vector. (Targeted for years 4-5.)

The toxin in the venom of *E. plathypenae* will be compared to that of *E. comstockii*. (Targeted for year 4.) If there are structural or host response differences then the toxin is to be characterized and the gene engineered into a baculovirus expression system. (Targeted for years 5 through FY 96).

INVESTIGATOR'S NAME(S):		J. J. Hamm and L. D. Chandler
AFFILIATION & LOCATION:		USDA, ARS, IBPMRL, Tifton, GA
ACTION AREA:	5	Biological Control
LEAD ARRAY:	5.1	Develop technology for managing *Heliothis/Helicoverpa* spp. using entomopathogens/nematodes
SAFEGD ARRAY:	5.1.1	Develop efficacious methods for mass producing entomopathogens/nematodes
OPTIM ARRAY:	5.1.2b	Develop formulation technology that will contribute to greatest field efficacy (entomo.); improve persistence to at least 7 days
OPTIM ARRAY:	5.1.2c	Develop application technology for optimum field efficacy (entom.) for particular regions of the country
SUPPL ARRAY:	5.1.3	Develop improved standard bioassays for determining bioactivity of entomopathogens.
DATES COVERED BY REPORT:		September 1991-July 1993

PROGRESS REPORT: The nematode *Steinernema riobravis* was field tested against corn earworm in silking corn in Georgia. A chemigation simulator was used to compare application of variable numbers of nematodes in 0.1 of water for control of corn earworm in corn silks and in soil. No progress was made in 5.1.1. A fluorescent brightener provided some UV protection to Heliothis virus. A bioassay technique was developed using cowpea seedling leaves to evaluate slow-acting formulations of Bacillus thuringiensis that allows for effects of sunlight on the pathogen.

FY94 & FY95 WORK PLANS: Continue testing *Steinernema riobravis* to determine its compatibility with the *Heliothis* NPV in an integrated pest management program. Compare application methods - irrigation rates, timing, etc., for *S. riobravis*. Further evaluate the B. t. bioassay technique with various formulations of B. t. and other entomopathogens.

INVESTIGATOR'S NAME(S):		C. M. Ignoffo
AFFILIATION & LOCATION:		USDA, ARS, BCIRL, Columbia, MO
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.1	Develop technology for managing *Heliothis/Helicoverpa* spp. using entomopathogens/nematodes.
SAFEGD ARRAY:	5.1.1	Develop efficacious methods for mass producing entomopathogens/nematodes
OPTIM ARRAY:	5.1.2a	Improve efficacy through natural or genetic manipulation of entomopathogens
OPTIM ARRAY:	5.1.2b	Develop formulation technology that will contribute to greatest field efficacy (entomo): improve persistence to at least 7 days
SUPPL ARRAY:	5.1.3	Develop improved standard bioassays for determining bioactivity of entomopathogens
DATES COVERED BY REPORT:		September 1990-June 1993

PROGRESS REPORT: The relative susceptibility of larvae of *Heliothis subflexa* to 4 feral, Australia isolates of HzSNPV was determined. Isolates of *Nomuraea* from different continents were tested.

A semi-defined medium was successfully used to culture several natural and mutant isolates of *Nomuraea rileyi*. Five strains of *M. anisopliae* were cultured *in vitro* on media containing gelatin, glucose plus nitrate or purified cuticle. Five strains of *B. bassiana* also were grown on defined media and a medium containing purified cuticle from *G. mellonella* or *T. ni*. Larval cuticle from *H. zea, H. virescens,* and *Trichoplusia ni* and a cellulose substrate were used to quantify release of proteolytic, chitinolytic, and lipolytic enzymes expressed by germinating conidia of *Nomuraea rileyi*.

Entomogenous fungi with pigmented conidia varying from black to white were exposed to simulated sunlight. Black conidia were significantly more stable than lighter pigmented conidia. Also, dry conidia were more stable than wetted conidia. Restriction fragment length polymorphisms were tested as markers for 11 strains of *Beauveria bassiana*. Some polymorphic differences appeared to correlate with virulence although a correlation between enzyme levels and virulence parameters (5 strains of *Metarhizium anisopliae)* was not apparent. A simple, relatively inexpensive starch-encapsulation process was used to formulate the nuclear polyhedrosis of with several sunlight-UV protectants (i.e. activated carbons, Tinopal, a fluorescent whitening agent, a dye (Congo Red) and a naturally derived polyflavanoid (Shade). A colorimetric system, with 19 substrates, was used to detect enzymes expressed by germinating conidia.

FY94 & FY95 WORK PLANS: Continue with studies to evaluate and identify entomopathogens that are potentially effective against the *Heliothis/Helicoverpa* complex. Continue studies on development and formulations of chemically defined media for growth and sporulation of entomopathogenic fungi. Continue with studies to improve efficacy of candidate microbial insecticides through natural, selective or genetic modification. Continue with studies to develop formulation and technologies for microbial insecticides to be used against larvae of the complex.

INVESTIGATOR'S NAME(S):		D. M. Jackson
AFFILIATION & LOCATION:		USDA, ARS, CRL, Oxford, NC
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.1	Develop technology for managing *Heliothis/Helicoverpa* ssp. using entomopathogens/nematodes
DATES COVERED BY REPORT:		September 1991-July 1993

PROGRESS REPORT: Various aspects of an autodissemination technique for the control of corn earworm, *Helicoverpa zea* (Boddie), larvae in sweet corn ('Silver Queen') have been investigated for three years (1991-1993) in large field tests at Oxford, NC and Lexington, KY. For this project we used a dust formulation of a baculovirus, *H. zea* nuclear polyhedrosis virus (*Hz*SNPV). This study is part or a Southern Regional IPM Project funded through the University of Kentucky (G. L. Nordin, principal investigator). There were four treatments (*Hz*SNPV treatment, powder control, insecticide control, and true control) with three replications of 1/4 acre sweet corn plots at each location. Pheromone-baited (with Zealure«) Texas-Style wire cone traps (1991) or baffled blacklight traps (1992 & 1993) were modified to accommodate a contamination station containing a walnut shell powder impregnated with the baculovirus, and a colored marking powder. Males passing through these stations were surface contaminated with the viral dust. Upon mating, they transferred some of this material to female moths, who subsequently transferred some virus to the eggs during oviposition. Some hatching larvae receive a lethal dose of virus when they chew through the egg chorion. Four pheromone-baited wire-cone monitor traps were placed around each field. Less than 10% of the moths captured in these traps were marked with fluorescent powder at either location. At Oxford in 1992, we achieved about 50% reductions in larval numbers, numbers of infested ears, and ear damage using this technique. For the other two seasons at Oxford, we achieved reductions of 20-30% for these parameters. No reductions in ear damage or larval numbers were observed for the Lexington, KY plots, probably due to later plantings with much higher corn earworm pressure. These data demonstrated the ability of refractive adult corn earworm moths to directly transmit *Hz*SNPV to F1 progeny by transovum transmission, and to indirectly enhance horizontal transmission of corn earworm larvae by passively dispersing virus onto the sweet corn crop. Up to 30% of male corn earworm moths passed through HzSNPV-contamination stations as estimated by mark and recapture methods. Bioassays of field-collected eggs confirmed that larvae died of *Hz*SNPV. Examination by SEM showed that many eggs had *Hz*SNPV polyhedra clustered on the upper hemisphere near the micropyle.

An epizootiological simulation model of the above described *Hz*SNPV autodissemination system was developed (primarily by G. C. Brown, University of Kentucky) to assist in an understanding of the epizootiological events, and to help guide further field research. This model indicated that the efficacy of the autodissemination technique is most sensitive to the trapping component of the male flight process.

INVESTIGATOR'S NAME(S):		A. H. McIntosh
AFFILIATION & LOCATION:		USDA, ARS, BCIRL, Columbia, MO
ACTION AREA	5.	Biological Control
LEAD ARRAY	5.1	Develop technology for managing *Heliothis/Helicoverpa* spp. using entomopathogens/nematodes
OPTIM ARRAY:	5.1.2a	Improve efficacy through natural or genetic manipulation of entomopathogens
SAFEGD ARRAY:	5.2.1b	Identify and characterize strains/species of native and introduced natural enemies of *Heliothis/Helicoverpa*
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Clones from two *Helicoverpa zea* (Lepidoptera: Noctuidae) ovarian cell lines, BCIRL-Hz-AM1 and BCIRL-Hz-AM3 were compared for their ability to produce OB and extracellular virus (ECV) when challenged with HzSNPV. Significant differences were found between the two parental cell lines and the five clones tested with regards to virus production. Two clones produced 4-5X as many OB as the parental cell line. The highest titers (PFU/ml) were also produced by the clones. These studies emphasize the value in cloning parental lines and then evaluating them for virus productivity.

Two baculoviruses, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and *H. zea* single nuclear polyhedrosis virus (HzSNPV) which infect the major pests *Heliothis virescens* and *H.zea* have been successfully replicated in insect cells grown in serum free media (SFM). Fetal bovine serum (SFM), a normal component of media, can represent as much as 75% of the cost of media. This accomplishment therefore represents a means of reducing cost production of these two baculoviruses and makes it more feasible for commercial production on a large scale. Both baculoviruses produced titers and occlusion bodies (OB) equivalent to or greater than that observed in serum containing media. Bioassays on the OB produced in cells grown in SFM were just as infectious for larvae as those produced in serum containing media.

A coleopteran cell line (AGE) derived from the cotton boll weevil, *Anthonomus grandis*, supported replication of AcMNPV producing approximately twice as many OB as a lepidopteran cell line (TN-CL1) derived from *Trichoplusia ni*. Occlusion bodies derived from both lines were equally infectious for *T. ni* larvae giving approximately the same LC_{50}. The AGE cell line was also the best cell line for the expression of the enzymes luciferase and *B*-galactosidase when compared with 8 lepidopteran cell lines challenged with an AcMNPV recombinant. Thus the AGE cell line appears to be the best cell line in stationary culture for production of OB and expression of the recombinant proteins under study.

A clonal isolate from a recently isolated baculovirus from the celery looper *Anagrapha falcifera* was evaluated for its ability to replicate in 8 different lepidopteran cell lines. A *Heliothis subflexa* cell line (BCIRL-HS-AM1) produced the highest titer ($10^{8.53}$ $TCID_{50}$/ml) whereas the other 5 permissive cell lines produced approximately the same concentration of OB. Two cell lines, *H. zea* and *H. armigera* were nonpermissive for the baculovirus. Information garnered from this study will aid in the suitable selection of the appropriate cell line for virus production.

FY94 & FY95 WORK PLANS: Research will continue into the development of new cell lines from *H. armigera* and *H. punctigera* (that occur in Australia) for the production of HzSNPV. Baculovirus recombinants will be produced *in vitro* by co-transfection of insect cell lines. Characterization and identification of baculovirus isolates (5.2.1b).

INVESTIGATOR'S NAME(S):		J. R. Raulston and H. E. Cabanillas
AFFILIATION & LOCATION:		USDA, ARS, CIRU, Weslaco, TX
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.1	Develop technology for managing Heliothis/Helicoverpa spp. using entomopathogens/nematodes
OPTIM ARRAY:	5.1.2c	Develop application technology for optimum field efficacy
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: An entomopathogenic nematode found parasitizing *Helicoverpa zea* prepupae and pupae in corn fields in the Lower Rio Grande Valley of northeast Mexico and south Texas has been described as a new species, *Steinernema riobravis*. Field surveys indicated this nematode was present in an average of 34% of the corn fields in the LRGV and accounted for about 50% of all prepupa and pupa mortality. Exposure of corn earworm prepupae to the nematode in laboratory bioassays indicated an LD_{50} at an exposure level of 13 infective juveniles/prepupa. One hundred percent mortality occurred at an exposure level of 100 infective juveniles/prepupa. To determine efficacy under field conditions, *S. riobravis* infective juveniles were applied to the soil surface in fruiting corn plots in 1991 and 1992. When nematodes were applied to the soil at a rate of 200,000/m^2 after 10% of the corn earworm larvae had exited the ear to pupate or when 50% had attained the last larval stage (large larvae), 95-100% parasitism was attained. Nematodes remaining in the soil up to 75 days post application resulted in 85% parasitism of corn earworm prepupae in the laboratory. In another field test, *S. riobravis* was compared to *S. carpocapsae* (all strain) for their ability to parasitize corn earworm prepupae. When *S. riobravis* was applied at a rate of 200,000/m^2, 95% parasitism occurred, however no parasitism occurred in plots receiving *S. carpocapsae*. The inability of *S. carpocapsae* was attributed to the high (>38° C) soil temperatures occurring during this experiment. When *S. riobravis* was applied to the soil through in-furrow irrigation, there was no significant difference in parasitism observed at application rates of 100,000 and 200,000 infective juveniles/m^2. Parasitism averaged 93.9 and 98.5% at the 100,000 and 200,000 application rates respectively. These data indicate the potential of *S. riobravis* for controlling corn earworm through soil application of the nematode. Suppression of *H. zea* in concentrated corn growing regions could greatly reduce the availability of migratory adults of this pest.

FY94 & FY95 WORK PLANS: New formulations of *S. riobravis* will be applied to the soil in vegetative and fruiting corn to determine their efficacy for reducing populations of corn earworm prepuae and pupae. Also formulas containing antidessicants will be tested for use against corn earworm larvae in whorl stage and on silking corn. Application technology research will be aimed at developing efficient delivery systems for the nematode including aerial application, in-furrow and center pivot irrigation application and subsurface application. Other research will be aimed at determining the residual efficacy of applied nematodes.

INVESTIGATOR'S NAME(S): J. L. Roberson

AFFILIATION & LOCATION: USDA, ARS, SFCIML, IRRU, Mississippi State, MS

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.1 Develop technology for managing *Heliothis/Helicoverpa* using entomopathogens/nematodes

SAFEGD ARRAY: 5.1.1 Develop efficacious methods for mass producing entomopathogens-nematodes

DATES COVERED BY REPORT: January 1992-December 1992

PROGRESS REPORT: Conducted large-scale rearing program to provide 1,000,000 *Heliothis zea* for virus production.

FY94 & FY95 WORK PLANS: Conduct large-scale rearing operations to provide 8,000,000 *Heliocoverpa zea* larvae for virus production.

INVESTIGATOR'S NAME(S):		P. V. Vail[a], T. J. Henneberry[b], and M. R. Bell[c]
AFFILIATION & LOCATION:		[a]USDA, ARS, HCRL, Fresno, CA; [b]USDA, ARS, WCRL, Phoenix, AZ; [c]USDA, ARS, SFCIML, Stoneville, MS
ACTION AREA:	5.	Biological control
LEAD ARRAY:	5.1	Develop technology for managing Heliothis/Helicoverpa spp. using entomopathogens/nematodes
OPTIM ARRAY:	5.1.2b	Develop formulation technology for greatest field efficacy; improve persistence to at least 7 days
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: The nuclear polyhedrosis virus, Af*M*NPV, has a broad host range and is infectious to *H. zea*, *H. virescens*, *Spodoptera exigua* and *Trichoplusia ni*. Susceptibility of *Pectinophora gossypiella* was demonstrated. In 1991 small field tests showed *H. zea* and *H. virescens* were approximately equal in susceptibility to residues of Af*M*NPV on cotton leaves. By day 7 less than 50% of the original activity towards *H. zea* was left on leaves when 4 x 10^{13} PIB/acre were applied. Residues of 4 x 10^{12} and 4 x 10^{11} PIB/acre caused less than 50% mortality 3 days after application. In 1991 similar field tests were conducted to compare Af*M*NPV and the *Heliothis* virus. Only *H. zea* larvae were used in these tests as they are slightly less susceptible to Af*M*NPV. Responses to the viruses were similar; less than 50% of the activity on leaves was left after 7 days for all doses tested. Results of these studies showed that Af*M*NPV has potential as a microbial control agent for control of both species.

In 1992 small field trials were conducted at all locations to further elucidate the potential of Af*M*NPV. Insects included *H. zea, H. virescens, S. exigua* and *T. ni*. Tests were conducted with a fluorescent brightener (M2R) to determine if field persistence increased. Time to 50% loss of original activity was extended from 5.5 to 11.5 days at the high Af*M*NPV rate with M2R. Tests in Arizona confirmed that all four species could be infected under field conditions. At Stoneville, MS the addition of M2R had no influence on field performance of Af*M*NPV. However, the concentration was probably too low. Af*M*NPV infectivity to *H. virescens* larvae was equal to or greater than nuclear polyhedrosis viruses isolated from the alfalfa looper and *Heliothis*. The addition of COAX in the Stoneville tests did not provide a clear pattern as to the relative merits of this adjuvant. Among the three locations the field infectivity of Af*M*NPV to the four species was demonstrated. During the period laboratory studies were conducted on potential enhancement of Af*M*NPV by M2R. Increases in Af*M*NPV activity of 7.8, 4.3, 2.9 and 13.6 fold were obtained when M2R was fed with Af*M*NPV to *T. ni, H. virescens, H. zea* and *S. exigua*, respectively. Concentrations from 0.25 to 1% provide the enhancement effect with *T. ni* larvae.

FY94 & FY95 WORK PLANS: Determine M2R concentrations required in field applications to provide enhancement effects observed in laboratory; determine persistence of Af*M*NPV and M2R under field conditions; determine infectivity of Af*M*NPV formulation; conduct laboratory and field persistence tests with formulations developed specifically for Af*M*NPV.

INVESTIGATOR'S NAME(S): J. E. Carpenter[a] and S. D. Pair[b]

AFFILIATION & LOCATION: [a]USDA, ARS, IBPMRL, Tifton, GA; [b]USDA, ARS, SCARL, Lane, OK

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.2 Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators

OPTIM ARRAY: 5.2.3b *In vitro* rearing of parasites and predators

DATES COVERED BY REPORT: September 1992-June 1993

PROGRESS REPORT: *Ichneumon* (=*Pterocormus*) *promissorius* (Erichson) (Hymenoptera: Ichneumomidae) is native to Australia where it has been collected from *Helicoverpa* spp. This pupal parasitoid searches the soil surface for host pupation sites, burrows into the pupal gallery, and oviposites into the host pupa. Studies have been initiated to examine the potential of *I. promissorius* as a biological control agent of pest species in the United States.

I. promissorius developed successfully on several native lepidopteran species including *Helicoverpa*, *Heliothis* species. Rearing *I. promissorius* in the laboratory was accomplished easily by exposing uncovered host pupae to female wasps. The developmental time of *I. promissorius* varied with the host species and the age of the host pupa at the time of oviposition.

Fecundity and rate of oviposition were influenced by the mating status of females, the host from which females developed, and the frequency in which females were exposed to hosts. Virgin females continued laying eggs many days after mated females had stopped laying eggs. As a result, more eggs were produced from virgin females (174.8 eggs/female) than from mated females (95.9 eggs/female). A preoviposition period of 17 days in mated females did not affect the oviposition curve or the number of eggs laid. These data suggest that oogenesis is arrested until female wasps are exposed to host pupae. Female wasps exposed to pupae for 24 h every 5th day lived longer than female wasps that were exposed to pupae continuously. However, female wasps that were exposed to pupae continuously laid more eggs. Virgin females reared on *Spodoptera exigua* (Hübner), pupae laid fewer eggs than virgin females reared on *Helicoverpa zea* (Boddie) pupae.

Releases of *I. promissorius* were initiated in a four-state effort during the summer of 1993. Data from these studies were not available at the time of this report.

FY94 & FY95 WORK PLANS: Studies on *I. promissorius* will emphasize reproductive and developmental biology, host preference and acceptance, foraging behavior, and diapause. Studies on the *In vitro* rearing of pupal parasitoids of *Heliothis/Helicoverpa* will be initiated.

In a four-state cooperative effort, *I. promissorius* will be released to determine: (1) its ability to colonize in the southern U.S.; (2) the influence of crop type and distance from the release site on colonization and parasitization rates and; (3) the ecological host range in various habitats. Cooperators: T. J. Kring, Univ. of AR; J. R. Raulston, USDA-ARS, Weslaco, TX.

INVESTIGATOR'S NAME(S):		T. A. Coudron
AFFILIATION & LOCATION:		USDA, ARS, BCIRL, Columbia, MO
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators
SAFEGD ARRAY:	5.2.1a	Develop stable efficacious methods for mass production and quality control and release methods for parasites/predators
SAFEGD ARRAY:	5.2.1b	Identify and characterize strains/species of native and introduced natural enemies of *Heliothis/Helicoverpa*
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Procedures have been developed and confirmed for the successful rearing of two parasitoids, *E. comstockii* and *Euplectrus plathypenae*. Both species are reported as parasites of *Heliothis/Helicoverpa* spp. Positive identification of both species was accomplished with the use of two simple and rapid chemical analyses, and confirmed with one morphological trait and mating analyses. Continuous colonies have been maintained for more than 100 generations (i.e., 9+ years). Handling methods were improved to extend the life span of the parasitoids to three times that reported in the literature. (Accomplished during years 1-2.) An adaptation of these procedures was used to mass rear *E. comstockii*. A production rate of >1,000/day was achieved by a one person rearing operation at an estimated cost of ca. 5 cents/female. The effectiveness of the mass reared *E. comstockii* was demonstrated in small field plot tests. (Accomplished during years 2-3.)

Funding from the Agricultural Experiment Station, University of Guam, has been received to collect, identify, and rear two species of *Euplectrus* that originated in India. The venom of these species will be tested for arrestment activity and compared to that of *E. comstockii*. (Targeted for year 4 and 5 through FY 97.)

INVESTIGATOR'S NAME(S):		S. M. Ferkovich
AFFILIATION & LOCATION:		USDA, ARS, IABBBRL, Gainesville, FL
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators
SAFEGD ARRAY:	5.2.1a	Develop stable efficacious methods for mass production and quality control and release methods for parasites/predators
SUPPL ARRAY:	5.2.3b	In vitro rearing of parasites and predators
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: *In Vivo* Rearing: Because of the extreme difficulty of developing an *in vitro* system for *Micropletis croceipes*, our research emphasis has been shifted toward *in vivo* rearing on atypical hosts. We have shown that four atypical hosts, *Spodoptera frugiperda, S. exigua, Trichoplusia ni, Plodia interpunctella* and *Galleria mellonella* which are less costly to rear than the typical hosts, *Heliothis virescens* and *Helicoverpa zea*, could be made attractive to females of *M. croceipes* by treating them with host frass and hemolymph. In the second phase of this study, we are determining the parasitoid's rate of development and the atypical host's immune response to the parasitoid egg. In *S. exigua*, 100% of the parasitoid eggs were encapsulated 3 days after oviposition, whereas in *S. frugiperda* a low rate of encapsulation initially occurred, allowing 13% to pupae and 12% to reach the adult stage. Parasitoid eggs were not encapsulated in *G. mellonella*; however, up to 77% of the 1st-3rd instar larvae were encapsulated. Of the unencapsulated parasitoid larvae, 24% pupated and 17% emerged as adults.

In Vitro Rearing: Successfully reared an endoparasitoid, *M. croceipes*, from the egg stage to the first larval instar *in vitro* using medium conditioned by cell lines. Also demonstrated that the cell lines released at least two egg developmental-promoting factors (germ band- and hatch-promoting), and that egg development was dependent upon the tissue source and insect species the cell line was derived from, and the composition of the culture medium. The results contribute toward *in vitro* rearing of an endoparasitoid in that one of the cell lines that supports *M. croceipes* development is already commercially mass produced for baculovirus production and thus opens up the potentiality of using the same technology to grow cell lines for mass rearing *M. croceipes*. These results also advance the progress of *in vitro* rearing in that they revealed to the incumbent and other scientists working in this area that optimizing the use of cell lines for *in vitro* rearing of *M. croceipes* will require the identification of growth factors from host hemolymph that are needed to promote development of the parasitoid beyond the first instar.

FY94 & FY95 WORK PLANS: *In Vitro* Rearing: Identify the germ band-promoting factor and the hatch-stimulating factor produced by insect cell lines and tissues that can be utilized in the following: (a) development of the first *in vitro* rearing technique for a Hymenopteran endoparasitoid, and (b) development of the first chemically defined culture media for insect cells and tissues using insect-derived growth factors.

In Vivo Rearing: We will investigate factors such as rearing temperature, host age, and treatment with radiation that may affect the encapsulation ability of a potential host species. These factors need to be investigated in order to find a means of inhibiting the encapsulation response of the alternate host. I also plan to develop a technique for studying the encapsulation process *in vitro*. This would afford taking hemocytes from potential host insects to predetermine whether or not they might be a suitable host without having to first rear the insects in the laboratory.

INVESTIGATOR'S NAME(S):		P. Greany
AFFILIATION & LOCATION:		USDA, ARS, IABBBRL, Gainesville, FL
ACTION AREA:	5.	Biological control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Heliocoverpa* spp. using parasites/predators
SUPPL ARRAY:	5.2.3b	*In vitro* rearing of parasites and predators
DATES COVERED BY REPORT:		September 1991-August 1993

PROGRESS REPORT: No progress to report (temporary suspension in this research activity during this period).

FY94 & FY95 WORK PLANS: Work on *in vitro* rearing of parasites of *Helicoverpa zea* and *Heliothis virescens* will resume (beginning in August 1993), but will no longer focus on *Microplitis croceipes*. Instead, attempts will be made to rear the following spp. of hymenopterous pupal parasites, *Ichneumon promissorius* & *Cryptus albitarsus*, and the dipteran larval parasite, *Archytas marmoratus* on artificial media.
This work will be performed in cooperation with Drs. Harry Gross and Jim Carpenter, USDA/ARS, Tifton, GA, and Dr. Allen Cohen, USDA/ARS, Phoenix, AZ.

INVESTIGATOR'S NAME(S):		M. H. Greenstone
AFFILIATION & LOCATION:		USDA, ARS, BCIRL, Columbia, MO
ACTION AREA	5.	Biological Control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators
SAFEGD ARRAY:	5.2.1a	Develop stable efficacious methods for mass production and quality control and release methods for parasites/predators
SAFEGD ARRAY:	5.2.1b	Identify and characterize strains/species of native and introduced natural enemies of *Heliothis/Helicoverpa*
SUPPL ARRAY:	5.2.3a	Improve efficiency and persistence of parasites/predators through chemical/behavioral ecology and genetic manipulation of parasites/predators
SUPPL ARRAY:	5.2.3c	Rearing of predators
DATES COVERED BY REPORT:		September 1991-1993

PROGRESS REPORT: A monoclonal antibody to *H. zea* arylphorin, which was the basis for a fifth-instar and species-specific (in the Western Hemisphere) immunoassay of predators fed fifth instars, was shown to recognize an antigenic determinant shared by *H. armigera* but not *H. punctigera*, which is in agreement with current systematic thinking on the heliothinae. This result may guide the selection of exotic predator species in the Old World.

The detectability of fifth instars in the gut of the paper wasp predator *Polistes metricus* held under field fluctuating temperature conditions was shown to decay exponentially, with a half-life of 19.4 h (5.2, year 1). This information is needed for the conversion of immunoassay data into quantitative predation estimates. Several species of hunting spiders of the families Lycosidae and Salticidae were characterized as to their genetic variability (5.2.1b, year 1).

An immunodot assay was developed which is able to detect a single heliothine egg in the gut of a predator. Predator homogenates may be spotted on nitrocellulose and stored up to six months before assay. Two grams of an anti-egg antibody have been provided to a foreign researcher for studies of egg predation on *H. armigera* in India (5.2.3c, years 2 and 3).

FY94 & FY95 WORK PLANS: Research will continue into the development of rapid, monoclonal antibody-based immunoassays for egg and larval identification and predator gut analysis, including exploration of new formats and media. Antibodies will be characterized as to species recognized, including *H. armigera*, and as to egg and larval antigenic determinants recognized. Egg and larval predators will be collected from field sites and assayed to determine whether they have fed upon *H. zea*. Studies of half-life of *H. zea* egg and larval antigen detectability will continue using additional predator taxa.

INVESTIGATOR'S NAME(S):		H. R. Gross
AFFILIATION & LOCATION:		USDA, ARS, IBPMRL, Tifton, GA
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators
SAFEGD ARRAY:	5.2.1a	Develop stable efficacious methods for mass production and quality control and release methods for parasites/predators
SUPPL ARRAY:	5.2.3a	Improve efficiency and persistence of parasites/predators through chemical/behavioral ecology and genetic manipulation of parasites/predators
SUPPL ARRAY:	5.2.3b	*In vitro* rearing of parasites/predators
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Inundative releases of *Archytas marmoratus* (Diptera:Tachinidae) at approximate rates of 1400 females per ha for three consecutive weeks against naturally occurring larval populations of *H. zea* in whorl-stage field corn in south Georgia, yielded mean rates of parasitization of 97.8 and 89.7% on two sampling dates, while naturally occurring parasitization by *A. marmoratus* in control fields was 19.1 and 5.8%. Opportunities for using inundatively released *A. marmoratus* in the integrated management of the F1 seasonal generation of *H. zea* in whorl-stage corn appear promising.

Advanced methods of mass propagating *A. marmoratus* on *Galleria mellonella* yielded approximately 42,000 adult parasitoids per week for inundative release, with a parasitoid:host recovery rate of approximately 70%.

FY94 & FY95 WORK PLANS: Efforts will continue to focus on improving the mass propagation efficiency of *A. marmoratus* on *G. mellonella*. Multiple methods of inundatively releasing *A. marmoratus* will be tested, including having parasitoids move directly into grower fields at time of emergence, and having them emerge into screened cages from which they will be released later as reproductively mature females. The relative effectiveness of single and multiple releases of selected densities of *A. marmoratus* will also be evaluated. The relative efficiency of AM when released inundatively against *H. zea* and *H. virescens* in cotton and other cultivated crops will also be determined.

INVESTIGATOR'S NAME(S): D. M. Jackson

AFFILIATION & LOCATION: USDA, ARS, CRL, Oxford, NC

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.2 Develop technology for managing *Heliothis/Helicoverpa* ssp. using parasites/predators

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Over the past six years (1988-1993) adult *Cardiochiles nigriceps* wasps, a parasitoid of tobacco budworm larvae, have been counted as they flew over replicated three-row plots of 117 accessions of 67 Nicotiana species (including tobacco, *Nicotiana tabacum*). *C. nigriceps* wasps are attracted to several Nicotiana species in the absence of their insect hosts. *N. noctiflora* and *N. sanderae* were especially attractive to C. nigriceps. Periodically over the last three years, wasps were netted as they flew over flowering tobacco, two accessions of *N. noctiflora*, and three accessions of *N. sanderae*. About 90% of the wasps flying over tobacco were females, while only about 25% of the wasps captured over *N. noctiflora* were females. Equal numbers of *C. nigriceps* were observed flying over flowering and non flowering (topped) *N. noctiflora* plants. Large quantities of leaf material from Nicotiana species that were most attractive to *C. nigriceps* were extracted with methylene chloride and frozen immediately on dry ice. These extracts are being used for the isolation of volatile components for bioassays against *C. nigriceps*. This is in cooperation with Drs. W. Schlotzhauer and R. F. Severson (USDA-ARS, Athens, GA).

INVESTIGATOR'S NAME(S):		W. J. Lewis[a] and J. H. Tumlinson[b]
AFFILIATION & LOCATION:		[a]USDA, ARS, IBPMRL, Tifton, GA; [b]USDA, ARS, IABBBRL, Gainesville, FL
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.2	Develop Technology for Managing *Heliothis/Helicoverpa* spp. using parasites /predators
DATES COVERED BY REPORT:		September 1991-July 1993

PROGRESS REPORT: The development of technology for managing *Heliothis/Helicoverpa* with natural enemies and other IPM practices was advanced along several lines: (1) Discovered that salivary secretions from H/H larvae feeding on plants trigger the plant to emit chemical signals vital to the host searching behavior of parasitoids and the demonstration that this attribute of the plant varies among varieties of the crop. This information is leading to the ability to select and engineer crop varieties that maximize the benefits of natural defenses. (2) Demonstrated the importance of adult food to effective performance of parasitoids; compared the effectiveness of food from different plants sources; assessed the value of receiving food at different times during rearing and release procedures; and determined the importance of chemical cues to food location. This information is leading to laboratory and field technology to enhance the abundance and effectiveness natural and released natural enemies. (3) Found that visual cues such as color and shape interacting with the chemical cues play a valuable role in the food and host foraging behavior of parasitoids. This information has expanded the prospects for manipulating and monitoring the foraging behavior of natural enemies. (4) Further elucidated the role and mechanisms of learning in host and food foraging by parasitoids. This information is leading to enhanced technology for the quality and performance of lab reared and feral natural enemies. (5) Determined the presence of three viruses in laboratory colonies of *M. croceip*es and their implications in biological control. This knowledge is important to the development of effective methods for production and release of quality parasitoids. (6) Developed and preliminarily tested a prototype semiochemically baited trap for live capture of foraging females of *M. croceipes*. This development in monitoring technology is leading to more effective use of natural and released natural enemies as an effective part of IPM strategies.

FY94 & FY95 WORK PLANS: Continue to field test various semiochemical and food spray formulations for enhancing the abundance and effectiveness of natural enemies. Use recent findings regarding the plant's emission of herbivore-induced foraging signals and adult food resources to select crop varieties that maximize the abundance and effective of natural enemies. Develop and evaluate cover crop and intercropping systems that provide food and foraging resources needed for fostering the effectiveness of natural enemies. Further develop semiochemically-baited traps and other tools for monitoring parasitoids as a part of a more comprehensive and sustainable means for pest management decision making.

INVESTIGATOR'S NAME(S):		D. A. Nordlund
AFFILIATION & LOCATION:		USDA, ARS, BCPRU, Weslaco, TX
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators
SAFEGD ARRAY:	5.2.1a	Develop stable efficacious methods for mass production and quality control and release methods for parasites/predators
DATES COVERED BY REPORT:		September 1991-July 1993

PROGRESS REPORT: Control of *Heliothis/Helicoverpa* in cotton by inundative releases of *Chrysoperla carnea* was demonstrated several years ago. However, high costs and limited production capacity have impeded the adoption of this potentially valuable management tool. Thus, efforts to improve the mass production systems for *Chrysoperla* spp. are under way.

Two improved egg harvesting systems have been developed. One relies on the use of a hot wire to cut the egg stalk (see Ridgway et al. 1977). This system required a new adult holding and oviposition unit that would unroll to permit egg harvest. While the system resulted in an improvement in egg harvest efficiency, there was no increase in the efficiency of adult holding. The second egg harvesting system is based on the use of a sodium hypochlorite solution. This harvesting system facilitated the development of adult holding and oviposition units with a high surface area to volume ratio, which significantly increased the efficiency of adult holding. The sodium hypochlorite based egg harvesting system could easily be automated.

A process for using hot melt glue to prepare larval rearing units was also developed. Use of hot melt glue is more labor efficient than traditional methods of unit preparation. Hot melt glue technology will permit the development of an automated system for preparation of larval rearing units using existing packaging technology. This would significantly increase production capacity and reducing per unit costs.
Finally, artificial diets were used extensively for larval rearing in our culture. Several artificial diets for *Chrysoperla* larvae have been developed over the years and procedures for encapsulating the liquid diet have been developed. We have used the Hassan and Hagen (1978) diet, provided via saturated cotton cloth, and a commercially available encapsulated diet (Bionova, Neuss, Germany). We are confident that artificial diet can replace much, if not all, of the natural prey generally used in rearing *Chrysoperla*. The use of artificial diet would significantly reduce production costs.

With an efficient automated rearing system for *Chrysoperla* spp. we should be able to effectively use these predators against a wide variety of pests, including *Heliothis/Helicoverpa*.

FY94 & FY95 WORK PLANS: Research emphasis will shift from the development of rearing technology to studies of field and greenhouse efficacy of *Chrysoperla*. Particular emphasis will be on the use of *Chrysoperla* for management of the sweetpotato whitefly (*Bemisia tabaci*).

INVESTIGATOR'S NAME(S):		J. L. Roberson
AFFILIATION & LOCATION:		USDA, ARS, SIML, IRRU, Mississippi State, MS
ACTION AREA:	5.	Biological Control
SAFEGD ARRAY:	5.2.1a	Develop stable, efficacious methods for mass production, quality control, release methods for parasites/predators
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Established production quality control procedures to include all phases of surface egg sterilization, diet preparation, and mechanized tray processes for *Heliothis* production.
Rearing equipment for oviposition room, oviposition cages, and egg washers were constructed to enable production of 700,000 *Heliothis virescens* backcross pupae per week and tested prior to initiation of insect production in March 1992. Produced 4.2 million pupae for 6-week release program.
Evaluated wax-coated cardboard box for dual purpose use as rearing containers and field release stations for *Micorplitis croceipes* parasites.

FY94 AND FY95 WORK PLANS: Refine system to improve monitoring sensitivity of microbial contamination.

INVESTIGATOR'S NAME(S):		W. W. M. Steiner
AFFILIATION & LOCATION:		USDA, ARS, BCIRL, Columbia, MO
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators
SAFEGD ARRAY:	5.2.1b	Identify and characterize strains/species of native and introduced natural enemies of *Heliothis/Helicoverpa*
SUPPL ARRAY:	5.2.3a	Improve efficiency and persistence of parasites/predators through chemical/behavioral ecology and genetic manipulation of parasites/predators
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Three promising flightless strains of *Microplitis croceipes* have been developed for biological control applications in patchy spring environments such as wild geranium, where spring *Heliothis/Helicoverpa* populations build density prior to invasion of cotton ecosystems. They maintain constant biological pressure against the developing moth larvae since their flightlessness prevents their leaving the release site. The flightless strains are currently undergoing genetic characterization and field plot testing to determine their effectiveness in different plant ecosystems under competition with each other and with other predators/parasites of the moth complex. It is anticipated that the field trials will lead to preparation and submission of a pilot test proposal in fall of 1993.

The following mutant strains of *M. croceipes* have been isolated and are in colony: Black abdomen (Bad), clear wing (clw), vestigial wing (vgw), clipped wing (clpw), vestigial wing with Black abdomen (vgi), clearwing with a bronze body (clwbr), fast electromorph of glutamate oxaloacetate transaminase (GOT-1^f), and slow or wild-type electromorph of glutamate oxaloacetate transaminase (GOT-1^s). Other mutants have been identified but have not yet been isolated. Most of these mutants have been isolated from a large collection of *M. croceipes* made near Kennett, Missouri and so have different genetic backgrounds from the Stoneville, MS derived stocks.

A lab colony of the braconid *Cardiocheles nigriceps* has been evaluated for 10 electrophoretic systems. Of these, four were found to be segregating for at least 2 alleles including an esterase (*EST-3*), a glutamate oxaloacetate transaminase (*GOT-1*), a leucine aminopeptidase (*LAP-1*) and a malate dehydrogenase (*MDH-1*). Field specimens of this species and of *Cotesia marginiventris* remain to be evaluated.

In addition to the above, the imported strain of *Microplitis demoliter* currently housed at Stoneville, MS has been electrophoretically characterized for 27 enzyme systems. The strain is fixed for a fast electromorph of *GOT-1* found in *M. croceipes* and shares the same electromorph at 8 other enzyme systems. Thus, the two species differ at 66.6% of their protein genes.

FY94 & FY95 WORK PLANS: To begin investigating the genetic basis of sex ratio and longevity in *Microplitis croceipes* and attempt to develop long-lived, all or mostly-female strains with various abilities to attack heliothids under field conditions.

INVESTIGATOR'S NAME(S): G. Tillman

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.2 Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators

DATES COVERED BY REPORT: June 1992-July 1993

PROGRESS REPORT: Native *Microplitis croceipes* parasitized 15% of the *Heliothis virescens* larvae on geranium in May of 1993. This parasitoid was the predominant species. Additive releases of this parasitoid in this early season plant may significantly reduce the second generation of hosts laying eggs in cotton. Factors affecting functional response models were evaluated in large field cages.

FY94 & FY95 WORK PLANS: Try to import an African *Cardiochiles* spp. which attacks *Heliocoverpa armigera* and evaluate the parasitoid's efficacy on *H. zea* and *H. virescens*. Evaluate parasitoid releases early-season versus during the growing season.

INVESTIGATOR'S NAME(S): J. H. Tumlinson and W. J. Lewis

AFFILIATION & LOCATION: USDA, ARS, IABBBRL, Gainesville, FL

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.2 Develop Technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators

LEAD ARRAY: 5.3 Biological Control, Develop IPM program, etc.

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: When *Heliothis/Helicoverpa* larvae feed on corn, cotton, soybean, or cowpea plants the plants actively produce/release volatile compounds. These compounds attract the parasitoid females of the species *Microplitis croceipes* and *Cotesia marginiventris* and thus aid them in finding their larval hosts. There are great differences in the volatile blends produced by different species of plants and thus the wasps need to learn in order to find their polyphagous hosts in a wide variety of chemical environments. The response by the plant is caused by a substance in the oral secretions of the larvae and the plants do not respond to artificial damage in the same way they respond to larval damage. The plant response is systemic and larval damage to one leaf causes the entire plant to emit volatile chemical signals. Different varieties of a plant species respond differently to larval damage. A wild variety of cotton produces about ten times the amount of volatiles as domestic varieties we have studied, when fed on by caterpillars. Some domestic varieties of cotton and corn produce very little volatile chemical signal in response to larval damage. This information should be useful in developing resistant crop varieties. Our results thus far and those of our colleagues in the Netherlands indicate that this phenomenon may be very general and part of the defense mechanisms used by plants.

FY94 & FY95 WORK PLANS: Identify factor in larval oral secretions that induces plants to emit chemical signals that attract parasitoids. Determine mechanism of action of this substance. Investigate several varieties of cotton and other plants to determine whether some varieties are more attractive to natural enemies of H/H than others and whether an attractive variety can be developed through breeding or gene transfer. Study the action of the active factor(s) from larval oral secretions on several species of crops and cover crops to determine which are likely to be most attractive to parasitoids and most likely to provide refuge for natural enemies. Investigate plant chemicals associated with location of food resources (e.g., extrafloral nectaries) for parasitoid wasps).

INVESTIGATOR'S NAME(S): G. W. Elzen

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.2 Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators

SAFEGD ARRAY: 5.2.1b Identify and characterize strains/species of native and introduced natural enemies of *Heliothis/Helicoverpa*

DATES COVERED BY REPORT: 1991-1993

PROGRESS REPORT: Sensory receptors on the antennae, labial and maxillary palpi, and foretarsi of *Microplitis croceipes* (Cresson) were examined by scanning electron microscopy. The occurrence of antennal sensilla not described by earlier workers was reported. Generally, there are 6 types of sensilla in adults of *M. croceipes*, namely, sensilla trichodea, s. basiconica, s. chaetica, s. placodea, s. campaniformia, and s. coeloconica. Sexual dimorphism in *M. croceipes* is correlated with antennal sensilla type. S. campaniformia are present only on female antennae whereas s. coeloconica and bent tipped trichoid sensilla occur only on male antennae.

FY94 & FY95 WORK PLANS: Evaluation of efficacy of *Nicotiana gossei* extract.

INVESTIGATOR'S NAME(S): J. J. Hamm[a], W. J. Lewis[a], W. W. M. Steiner[b]

AFFILIATION & LOCATION: [a]USDA, ARS, IBPMRL, Tifton, GA; [b]USDA, ARS, BCIRL, Columbia, MO

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.2 Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators

SUPPL ARRAY: 5.2.3a Improve efficiency and persistence of parasites/predators through chemical/behavioral ecology and genetic manipulation of parasites/predators

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: Colonies of *Microplitis croceipes* were screened by electron microscopy for viruses which might limit the productivity of the parasitoid colonies. A nonoccluded baculovirus was associated with early mortality of wasps. A selection process was developed which reduced the incidence of the virus in the colony. A small icosahedral virus was found in all colonies examined and may be associated with mortality of parasitoid larvae since it appeared to be more abundant in larvae that failed to spin cocoons or spun cocoons but failed to pupate. A reo-like virus was found in some colonies of *M. croceipes* but has not yet been associated with increased mortality or reduced productivity.

FY94 & FY95 WORK PLANS: Continue screening colonies of parasitoids and predators of *Heliothis/Helicoverpa* for pathogens, especially if there appear to be problems in maintaining production of colonies.

INVESTIGATOR'S NAME(S):		G. Tillman
AFFILIATION & LOCATION:		USDA, ARS, SFCIML, Stoneville, MS
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.3	Develop an IPM program emphasizing use of biocontrol practices and preservation of natural enemies in integrated systems
OPTIM ARRAY:	5.3.2	Improve rearing methods for host material for pathogens and for parasites/predators
SUPPL ARRAY:	5.3.3	Protection of successive crops by early application of entomopathogens and beneficial arthropods
DATES COVERED BY REPORT:		June 1992-July 1993

PROGRESS REPORT: In the laboratory, *M. croceipes* did not prefer *H. virescens* larvae over *Heliothis* backcross larvae. No difference in fecundity and development of immatures of *M. croceipes* occurred between *H. virescens* larvae and *Heliothis* backcross larvae. Field rates of parasitization by *M. croceipes* were not different for *H. virescens* larvae and *Heliothis* backcross larvae.

A bacterium lethal to *M. croceipes* in low numbers was identified. Rearing efficiency for this parasitoid was increased by using an antibiotic.

A "release box" has been designed for releasing parasitoids with a reduction in labor, money and space. A method of using cold storage to accumulate large numbers of parasitoids for release at the time when the preferred host larvae are available in the field is being refined. The efficacy of parasitoids released from the "release boxes" is being compared to that of parasitoids which have been reared in cages as in the past. Sesame was used as a trap crop for *Helicoverpa zea* and *H. virescens*. These pests preferred ovipositing eggs in sesame. The sesame was also a nursery for the naturally occurring parasitoids.

FY94 & FY95 WORK PLANS: Evaluate the effect of crimson clover as a cover crop on parasitization of *H. zea* and *H. virescens* by native parasitoids in cotton.

Evaluate the effect of a nursery with a seasonal succession of host plants on parasitization of *H. zea/H. virescens* in cotton.

Continue to improve the technique of rearing parasitoids in a "release box."

INVESTIGATORS' NAME(S): W. J. Lewis[a], J. H. Tumlinson[b], and J. J. Hamm[a]

AFFILIATION & LOCATION: [a]USDA, ARS, IBPMRL, Tifton, GA; [b]USDA, ARS, IABBBRL, Gainesville, FL

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.3 Develop an IPM program emphasizing use of preservation of natural enemies as part of integrated systems

DATES COVERED BY REPORT: September 1991-July 1993

PROGRESS REPORT: The development of technology for managing *Heliothis/Helicoverpa* with natural enemies and other IPM practices were advanced along several lines: (1) Discovered that salivary secretions from *Heliothis/Helicoverpa* larvae feeding on plants trigger the plant to emit chemical signals vital to the host searching behavior of parasitoids and the demonstration that this attribute of the plant varies among varieties of the crop. This information is leading to the ability to select and engineer crop varieties that maximize the benefits of natural defenses. (2) Demonstrated the importance of adult food to effective performance of parasitoids; compared the effectiveness of food from different plants sources; assessed the value of receiving food at different times during rearing and release procedures; and determined the importance of chemical cues to food location. This information is leading to laboratory and field technology to enhance the abundance and effectiveness natural and released natural enemies. (3) Determined the role and importance of a chemical marker pheromone in the foraging behavior of *Microplitis croceipes*. This knowledge is important to understanding factors influencing the effective on natural enemies in IPM systems. (4) Determined the presence of three viruses in laboratory colonies of *M. croceip*es and their implications in biological control. This knowledge is important to the development of effective methods for production and release of quality parasitoids. (5) Developed and preliminarily tested a prototype semiochemically-baited trap for live capture of foraging females of *M. croceipes*. This development in monitoring technology is leading to more effective use of natural and released natural enemies as an effective part of IPM strategies. (6) Determined the potential joint benefit of the integrated use of sublethal levels of Bt with parasitoids. This development is leading to enhanced use of these entomopathogens and similar biopesticides as an effective tool that intermeshes harmoniously with other parts of an IPM system.

FY94 & FY95 WORK PLANS: Continue to field test various semiochemical and food spray formulations for enhancing the abundance and effectiveness of natural enemies. Use recent findings regarding the plant's emission of herbivore-induced foraging signals and adult food resources to select crop varieties that maximize the abundance and effective of natural enemies. Develop and evaluate cover crop and intercropping systems that provide food and foraging resources needed for fostering the effectiveness of natural enemies. Evaluate sublethal dosages of *B. t.* and other biorationals as tools for complimenting natural enemies and other pest management strengths inherent in cropping systems. Further develop semiochemically-baited traps and other tools for monitoring parasitoids as a part of a more comprehensive and sustainable means for pest management decision making.

INVESTIGATOR'S NAME(S):		M. R. Bell
AFFILIATION & LOCATION:		USDA, ARS, SFCIML, Stoneville, MS
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.3	Develop an IPM program emphasizing use of biocontrol practices and preservation of natural enemies
SUPPL ARRAY:	5.3.3	Protection of successive crops by early application of entomopathogens and beneficial arthropods
DATES COVERED BY REPORT		September 1991-June 1993

PROGRESS REPORT: Two large area field trials were conducted in which early season hosts of bollworm/budworm were treated by aircraft using *Hz*SNPV at a rate of 240 billion polyhedra/acre to determine the effect on numbers of moths emerging and moving to crop hosts. Pheromone trap data from the first test (64,000 acres treated) showed that the population of emerging moths was reduced by 19-38% Deposition of the virus in that test was shown to be ~ 85% less than in an earlier test where the virus was sprayed by hand and by aircraft. This reduction was speculated to have been caused by wind and evaporation, since the treatment was applied from a higher altitude. A second large area test was treated using an evaporation retardant plus pattern spraying to improve coverage. Four aircraft were used to treat ~ 17,106 acres at a rate of 240 billion polyhedra/acre. Emergence data from cages placed over treated and untreated areas indicated that virus treatments reduced budworm emergence by 80.6 % and bollworm emergence by 46.2%. During the emergence period, budworm traps in the center 2 mile diameter of the treated area captured an average of 6.4 moths/trap/night compared with 11.4 moths/trap/night in the area surrounding the treated area, a reduction of 43.9%. Trap counts of bollworm adults during that period indicated a 21% reduction in the center 2 mile diameter.

FY94 & FY95 WORK PLANS: Effectiveness of celery looper virus in reducing budworm and bollworm emergence from early season weed hosts will be evaluated. Through cooperative efforts (and after background studies), a planned test area of ~ 400 square miles will be treated with NPV to determine the efficacy of early season control as a management program for use against *Heliothis/Helicoverpa* in the delta. This will also require the development of procedures to produce the virus needed. Other small plot field studies will be conducted to evaluate new candidate NPVs for use in control programs.

INVESTIGATOR'S NAME(S): C. M. Ignoffo

AFFILIATION & LOCATION: USDA, ARS, BCIRL, Columbia, MO

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.3 Develop an IPM program emphasizing use of biological control practices and use of natural enemies

SAFEGD ARRAY: 5.3.1 Field assessment and fate of artificially applied/released entomopathogens and parasites/predators.

PROGRESS REPORT: Inclusion bodies of the HzSNPV exposed in water to simulated sunlight (SUV) were about 3 times more sensitive than dry PIB. pH (at 3, 6, or 9) had no significant effect on inactivation of PIB by SUV. Temperature of exposure (10, 22, 35, or 50°C) also had no effect on PIB exposed to SUV. Normally encountered field temperatures or pH, coupled with exposure to sunlight, should not adversely affect stability of HzSNPV. Presence of free water, however, can significantly increase the PIB inactivation by sunlight.

FY94 & FY95 WORK PLANS: Continue studies to assess environmental impact on entomopathogens that are potential candidates for use as microbial insecticides.

INVESTIGATOR'S NAME(S): [a]W. C. Nettles, Jr., [a]G. Saldaña, [b]Z. N. Xie, [b]Z. X. Wu, and [c]G. Feng

AFFILIATION & LOCATION: [a]USDA, ARS, BCIPRU, Weslaco, TX; [b]Texas A&M University; [c]USDA, OICD

ACTION AREA: 5. Biological Control

LEAD ARRAY: 5.2 Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators

SUPPL ARRAY: 5.2.3b *In vitro* rearing of parasites and predators

DATES COVERED BY REPORT: July 1992-July 1993

PROGRESS REPORT: About 18 months ago our research reached the level that allowed us to conclude that economical *in vitro* mass production of *Trichogramma* spp. was likely. We have continued to make excellent progress in the past year and are close (probably as little as one to two years away) to being able to mass-produce *Trichogramma* on artificial diets. Potential patents prevent us from disclosing specific information.

FY94 & FY95 WORK PLANS: To continue to develop improved and economical artificial media, etc. for *Trichogramma* spp.

INVESTIGATOR'S NAME(S):		[a]W. C. Nettles, Jr., [b]A. Bratti, [b]E. Mellini
AFFILIATION & LOCATION:		[a]USDA, ARS, BCIPRU; [b]Istituto di Entomologia, Universita di Bologna, Bologna, Italy
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.2	Develop technology for managing *Heliothis/Helicoverpa* spp. using parasites/predators
SUPPL ARRAY:	5.2.3b	*In vitro* rearing of parasites and predators
DATES COVERED BY REPORT:		July 1991-July 1993

PROGRESS REPORT: When *Eucelatoria bryani* and *Palexorista laxa* are reared *in vitro* on agar-containing, host-based artificial diets, yields average as high as 80% adults. This is very significant because it demonstrates that our diet presentation techniques are satisfactory (agar based diets are not harmful to tachinids which recently were shown to have blind guts). Because high yields can be obtained *in vitro* on host based diets, it is important that we identify host factors needed for parasitoid growth and development. A large amount of significant tachinid research (an offshoot of the Nettles program) is being conducted at the University of Bologna.

FY94 & FY WORK PLANS: To continue to develop improved and economical artificial media, etc. for several species of Tachinidae.

INVESTIGATOR'S NAME(S):		F. C. Tingle, E. R. Mitchell, and J. R. McLaughlin
AFFILIATION & LOCATION:		USDA, ARS, IABBBRL, Gainesville, FL
ACTION AREA:	5.	Biological Control
LEAD ARRAY	5.3	Develop an IPM program emphasizing use of biocontrol practices and preservation of natural enemies in decision making as part of integrated systems
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Larval populations of *Heliothis virescens*, *Helicoverpa zea*, *Spodoptera frugiperda*, *S. exigua* and *Pseudoplusia includens*, were monitored in late-season cotton in an area of Northcentral Florida where cotton has not been grown in many years. Parasitization levels and species identification were determined throughout the season from the emergence of parasitoids from field-collected larvae of each pest species. It is important that we study the parasitoid populations so that we can develop methods to preserve the native populations of beneficial insects, whether conventional methods or pheromones for mating disruption are used for control of the pest species.

A 45 acre dry-land field, planted June 6, 1992 as the first crop of the year, and a 380 acre irrigated field that was planted July 11-18 following an early corn crop were used in the first year of the study. Prior to September 1, the predominant pest species in the earlier planted cotton was fall armyworm. Soybean looper then became the most common pest until the cotton matured. In cotton that followed early corn, tobacco budworm was the predominant species until replaced by fall armyworm in late August. The soybean looper population peaked in mid-September, but was outnumbered by beet armyworm in late October.

At least 12 species of parasitoids emerged from the lepidopterous larvae collected from the cotton plants. The primary parasitoid of tobacco budworm, corn earworm, fall armyworm, and beet armyworm in each field was *Cotesia marginiventris*. This species made up 66% of the total parasitoids from this group of larvae in the dry-land cotton and 83% of those from the irrigated field that was planted after corn harvest. The second most common parasitoid in each field was *Meteorus autographae* (10 and 15% in the dry-land and irrigated fields, respectively).

Cotesia marginiventris also was the major parasitoid that emerged from soybean looper larvae in the irrigated field and made up 67% of the total parasitoids. However, 92% of the parasitized soybean loopers in the earlier planted cotton contained *Copidosoma truncatellum*.

FY94 & FY95 WORK PLANS: In the field, evaluate effect on native parasitoid populations of pheromone treatments used in cotton for mating disruption of tobacco budworm, corn earworm, fall armyworm and beet armyworm. In cage studies, investigate effect of biological pesticides on the parasitoid, *Cotesia marginiventris*.

INVESTIGATOR'S NAME(S):		H. R. Gross
AFFILIATION & LOCATION:		USDA, ARS, IBPMRL, Tifton, GA
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.3	Develop an IPM program emphasizing use of biocontrol practices and preservation of natural enemies in decision-making as part of integrated systems
OPTIM ARRAY:	5.3.2	Improve rearing methods for host material for pathogens and for parasites/predators
SUPPL ARRAY:	5.3.3	Protection of successive crops by early application of entomopathogens and beneficial arthropods.
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Parasitization responses of *A. marmoratus* (Diptera:Tachinidae) to *Galleria mellonella* was critically dependent on the diet ingredients on which *G. mellonella* larvae developed. *A. marmoratus* allowed to develop on *G. mellonella* reared on the diet of Dutky et al. (1962) yielded only 31.2% recovery of adult parasitoids, while those reared on the diet of King et al. (1979) yielded 90.7% adults. Neither the Polyvisol Vitamin Mix nor sucrose when incorporated at four rates in the *G. mellonella* diet developed by King et al. (1979) had any significant effect on the percentage of adult *A. marmoratus* recovered, or the weight of resulting adult males or females. All four rates of Torula yeast incorporated in the GM diet, yielded significantly heavier *A. marmoratus* adults than did diets that did not include Torula yeast. When bee hive foundation wax was incorporated in the *G. mellonella* diet the weight of adult male parasitoids increased, but the weight of adult females did not. Data suggest that the size and percentage recovery of *A. marmoratus* adults can be increased, as the association between host diet and parasitoid production is better understood.

FY94 & FY95 WORK PLANS: Efforts will continue to focus on improving the efficiency and cost effectiveness of diets used to rear *G. mellonella* as a host for the propagation of *A. marmoratus*.

INVESTIGATOR'S NAME(S):		M. H. Greenstone
AFFILIATION & LOCATION:		USDA, ARS, BCIRL, Columbia, MO
ACTION AREA	5.	Biological Control
LEAD ARRAY:	5.3	Develop an IPM program emphasizing use of biocontrol practices and natural enemies as part of integrated systems
SAFEGD ARRAY:	5.3.1	Field assessment and fate of artificially applied/released entomopathogens and parasites/predators
SUPPL ARRAY:	5.3.3	Protection of successive crops by early application of entomopathogens and beneficial arthropods
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: A monoclonal antibody to *H. zea* arylphorin, which was the basis for a fifth-instar and species-specific (in the Western Hemisphere) immunoassay of predators fed fifth instars, was shown to recognize an antigenic determinant shared by *H. armigera* but not *H. punctigera*, which is in agreement with current systematic thinking on the heliothinae. This result may guide the selection of exotic predator species in the Old World.

The detectability of fifth instars in the gut of the paper wasp predator *Polistes metricus* held under field fluctuating temperature conditions was shown to decay exponentially, with a half-life of 19.4 h (5.2, year 1). This information is needed for the conversion of immunoassay data into quantitative predation estimates. An immunodot assay was developed which is able to detect a single heliothine egg in the gut of a predator. Predator homogenates may be spotted on nitrocellulose and stored up to six months before assay. Two grams of an anti-egg antibody have been provided to a foreign researcher for studies of egg predation on *H. armigera* in India (5.2.3c, years 2 and 3).

FY94 & FY95 WORK PLANS: Research will continue into the development of rapid, monoclonal antibody-based immunoassays for egg and larval identification and predator gut analysis, including exploration of new formats and media. Antibodies will be characterized as to species recognized, including *Helicoverpa armigera*, and as to egg and larval antigenic determinants recognized. Egg and larval predators will be collected from field sites and assayed to determine whether they have fed upon *H. zea*. Studies of half-life of *H. zea* egg and larval antigen detectability will continue using additional predator taxa.

INVESTIGATOR'S NAME(S):		J. L. Roberson
AFFILIATION & LOCATION:		USDA-ARS-SIML, IRRU, Mississippi State, MS
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.3	Develop an IPM program emphasizing use of biocontrol practices and preservation of natural enemies in integrated systems
OPTIM ARRAY:	5.3.2	Improve rearing methods for host material for pathogens and parasites/predators
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Identified a pathogenic bacteria infecting *Microplitis croceipes* rearing containers. The bacteria was controlled by adding antibiotic to the larval diet.

FY94 & FY95 WORK PLANS: Establish production backup procedures for maintenance colonies.

INVESTIGATOR'S NAME(S):		J. J. Hamm, J. E. Carpenter and L. D. Chandler
AFFILIATION & LOCATION:		USDA, ARS, IBPMRL Tifton, GA
ACTION AREA:	5.	Biological Control
LEAD ARRAY:	5.3	Development of IPM programs emphasizing use of biocontrol practices and preservation of natural enemies in decision-making as part of integrated systems
OPTIM ARRAY:	5.3.2	Improve rearing methods for host material for pathogens and for parasites/predators
SUPPL ARRAY:	5.3.3	Protection of successive crops by early application of entomopathogens and beneficial arthropods
DATES COVERED BY REPORT:		September 1991-July 1993

PROGRESS REPORT: Discovered gonadal dysgenesis in the *Helicoverpa zea* colony at Stoneville, Mississippi which limited the productivity of the colony.

Application of Elcar, *Heliothis* NPV, to field corn through the center pivot irrigation system significantly increased mortality of corn earworm larvae due to NPV and reduced the number of corn earworm moths captured in emergence cages in the treated plots. The projected number of moths produced per acre in untreated plots was 1,518 in 1991 and 2,698 in 1992. The reduction in number of moths produced varied from 25 to 100% depending on timing and rate of application. Elcar was most effective in reducing the number of moths produced when the corn was treated once during the tassel stage and three times during the silking stage.

FY94 & FY95 WORK PLANS: Continue work on gonadal dysgenesis in *H. zea*. Continue work on effects of timing and number of applications of Elcar to corn on number of corn earworm moths produced. Expand to larger acreage using chemigation technology. Evaluate *Steinernema riobravis* for early season control of *H. zea* on corn and study interactions between *S. riobravis* and other entomopathogens, especially NPV.

TABLE 5. Summary of Research Progress for Action Area V, Biological Control, in Relation to Year 2 Goals of the 5-Year Plan.

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
5.1 Develop technology for managing *Heliothis/ Helicoverpa* spp. using entomopathogens/ nematodes.	Identify entomopathogens for use in pest management systems, conduct laboratory and field experiments to examine effectiveness, and evaluate the compatibility of individual entomopathogens with various crop production practices.	X		Significant progress was made in selecting pathogens and developing technology to produce pathogens. *In vivo* production of HzSNPV, HaMNPV, and AfMNPV was demonstrated. Cell lines for *in vitro* production of these viruses were improved. Preliminary progress was made in improving efficacy of viruses and fungi: a toxin from *Euplectrus* venom lowered the LC50 of AcMNPV. Significant progress was made in increasing virus efficacy through UV protection and/or enhanced infectivity: the fluorescent brightener M2R increased activity of AvMNPV. Significant progress was made in application of entomopathogens aerially, in irrigation, and by autodissemination. Little progress was made toward standardizing bioassays of entomopathogens.
5.2 Develop technology for managing *Heliothis/ Helicoverpa* spp. using parasites/predators.	Conduct field studies with native and imported parasitoids and predators, consider pesticide treatments.	X		Releases of *A. marmoratus* at 1400 females/ha resulted in up to 97.8% parasitism of *H. zea*. A flightless strain of *M. croceipes* and the imported *I. promissorius* are being evaluated in the field. Procedures were developed for rearing two *Euplectrus* species. *M. crociepes* was reared on atypical hosts and 3 viruses were isolated from lab colonies of *M. crociepes*. A wax coated cardboard box was used for both rearing and field release of *M. crociepes*. Three flightless mutant strains have been isolated and are in colony. Several species of hunting spiders were genetically characterized. *I. promissorius* was imported from Australia and can be reared on *Heliothis/ Helicoverpa* pupae. Salivary secretion from *Heliothis/ Helicoverpa* larvae triggered plants to emit chemicals used in host searching by parasitoids. Visual cues also played an important role. An immunodot assay will detect a single heliothine egg in the gut of a predator.

TABLE 5 - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
5.3 Develop an IPM program emphasizing use of biocontrol practices and preservation of natural enemies in decision-making as part of integrated systems.	Develop plans for implementation, Identify and select specific practices.	X		Some progress has been made and considerable work planned to determine the compatibility of various approaches to control *Heliothis/Helicoverpa*. Little progress was made in determining criteria for evaluating efficacy of artificially applied biocontrol agents or determining suppression level feasible. Significant progress was made in rearing host larvae for *A. marmoratus* and limiting factors in the mass rearing of *H. zea* were identified. Excellent progress was made in applying biotic agents to early season crops to suppress populations, however the evaluation of carry over of impact is lagging. Plans for large scale tests should help in this area.

RESEARCH SUMMARY: ACTION AREA V—BIOLOGICAL CONTROL

Compiled by J. J. Hamm and P. G. Tillman

The *Heliothis/Helicoverpa* working conference and National Action Plan of 1991 identified three major areas for research in biological control of *H. virescens* and *H. zea*. These three areas are: (1) develop technology for using entomopathogens and nematodes, (2) develop technology for using parasites and predators, and (3) develop an IPM program emphasizing use of biocontrol practices and preservation of natural enemies in decision-making as part of integrated systems.

LEAD ARRAY 5.1: Considerable progress has been made toward identifying entomopathogens considered most efficacious for field use in pest management systems and evaluating the compatibility of individual entomopathogens with various crop production practices as evidenced by the following accomplishments: Screened isolates of *Nomuraea* from different geographical areas. Screened 4 feral isolates of HzSNPV against *H. subflexa* and *H. zea*. Determined that AfMNPV is ca. equally effective against *H. zea* and *H. virescens* and that it also infected *Spodoptera exigua* and *Trichoplusia ni* in field tests. Described a new species of nematode, *Steinernema riobravis*, found attacking *H.zea* prepupae and pupae. Determined that *S. riobravis* was more effective than *S. carpocapsae* in field tests.

Significant progress has been made in the selection of specific pathogens considered potential candidates for future production and in the technology needed for production of entomopathogens: Conducted large scale rearing program to provide 1,000,000 *H. zea* for virus production. Demonstrated capability of *in vivo* production of HzSNPV, HaMNPV, and AfMNPV. Improved cell lines for *in vitro* production of HzSNPV, AcMNPV and AfMNPV. Selected clonal isolate of AfMNPV for improved *in vitro* production. Improved media for production of *Nomuraea rileyi*.

Preliminary progress has been made in identifying some potential means of improving efficacy of entomopathogenic viruses and fungi: Isolated toxin form venom of a *Euplectrus* and studied its interaction with 2 viruses; the toxin lowered the LC50 of AcMNPV. Screened 16 venoms against *H. zea* larvae.

Significant progress has been made in the identification of materials with potential to increase the efficacy of entomopathogenic virus through UV protection and/or enhanced infectivity: In laboratory studies the fluorescent brightener M2R increased the activity of AfMNPV in *Trichoplusia ni, H. virescens, H. zea*, and *Spodoptera exigua*. A starch-encapsulation process was used to formulate HzSNPV with several UV protectants to improve activity of the virus.

Significant progress has been made in demonstrating the feasibility of applying entomopathogens aerially, in irrigation, and by autodissemination.

Little progress has been made toward standardization of bioassays of entomopathogens.

LEAD ARRAY 5.2: Some progress has been made toward field evaluation of native and imported species of parasitoids. Inundative releases of *A. marmoratus* at approximate rates of 1400 females per ha for 3 consecutive weeks against *H. zea* resulted in 97.8% and 89.7% parasitization for two sampling dates. A flightless strain of *M. croceipes* and the imported *I. promissorius* are both being evaluated in the field. Little progress has been made, but considerable work is planned for testing the feasibility of parasitoid releases early-season versus during the growing season.

Some progress has been made and considerable work is planned in developing methods for mass production of parasitoids and predators. Procedures have been developed for rearing two *Euplectrus* species. Advances were

made on rearing technology for *C. carnea*. Demonstrated ability to rear *M. croceipes* on atypical hosts to reduce rearing costs. Determined the presence of three viruses in laboratory colonies of *M. croceipes*.

Little progress has been made toward developing quality control in mass production of parasitoids.

Some progress has been made toward developing release methods for parasitoids. Demonstrated efficiency of wax-coated cardboard box dual purpose use as rearing containers and field release stations for *M. croceipes*, reducing mass rearing costs.

Established production quality control procedures for *Heliothis* production.

Considerable progress has been made toward identifying and characterizing strains/species of native and introduced natural enemies of *Heliothis/Helicoverpa*. Three flightless mutant strains of *M. croceipes* have been isolated and are in colony. Several species of hunting spiders were genetically characterized. *I. promissorius* has been imported from Australia and can easily be reared on *Heliothis/Helicoverpa* pupae. Plans have been made to find and import an African *Cardiochiles* spp. and two species of *Euplectrus* from India.

Considerable progress has been made in improving the efficiency and persistence of parasitoids through chemical and behavioral ecology of parasitoids. Discovered that salivary secretion from *Heliothis/Helicoverpa* larvae feeding on plants trigger the plant to emit chemical signals vital to the host searching behavior of parasitoids. Demonstrated the importance of adult food to effective performance of parasitoids. Found that visual cues such as color and shape interacting with chemical cues play a valuable role in the food and host-foraging behavior of parasitoids. Preliminarily tested a prototype semiochemically-baited trap for live capture of foraging females of *M. croceipes*.

Little further progress has been made toward *in vitro* rearing of two hymenopterous pupal parasitoids and one dipteran larval parasitoid.

Some progress has been made in developing immunological methods to identify prey consumption by P. metricus. An immunodot assay was developed with is able to detect a single heliothine egg in the gut of a predator.

LEAD ARRAY 5.3: Some progress has been made and considerable work is planned to determine the compatibility of various approaches to control *Heliothis/Helicoverpa*.

Little progress has been made toward determining criteria for evaluating efficacy of artificially applied/released biocontrol agents or determining suppression level feasible.

Significant progress has been made in rearing host larvae for *Archytas marmoratus* and limiting factors in the mass rearing of *H. zea* were identified.

Excellent progress has been made in the application of biotic agents to early season crops to suppress populations of *Heliothis/Helicoverpa*; however, the evaluation of carry over of impact to subsequent crops is lagging. Plans for large scale tests should help in this area.

BREAKOUT SESSION SUMMARY

The new emphasis on area-wide management of insect pests was discussed. The general consensus was that much of our research in biocontrol should fit well into an area-wide approach. The large tests with Elcar applied to early-season host plants for control of first generation *Heliothis/Helicoverpa* are a prime example.

It was recognized that where significant areas of corn are grown (2-3 million ha in 10 southern states) the generation of *H. zea* produced on whorl- and ear-stage corn is a key factor in population management because of the protected niche that corn offers. While parasitoids such as *Archytas marmoratus* have potential for reducing the subsequent population of *H. zea* on whorl-stage corn, the best candidates for reducing the population in ears of corn appear to be the *Heliothis* nuclear polyhedrosis virus applied to the silks to infect larvae as they enter the ear, or the nematode *Steinernema riobravis* applied to the soil to infect mature larvae as they enter the soil to pupate. These approaches would be compatible with other approaches such as Bt-transgenic cotton, hybrid sterility in *H. virescens*, or inherited sterility in *H. zea*.

The use of specific management strategies directed against specific generations of the pests would help to prevent the development of resistance to any of the control agents.

Year-round conservation of natural enemies and early season augmentation of natural enemies can be important approaches. Successful augmentation programs require continued research on mass rearing and quality control of both host insects and natural enemies.

Action Area VI. GENETICS, MOLECULAR BIOLOGY, AND BASIC PHYSIOLOGY

Coordinators: J. E. Carpenter and A. C. Bartlett

INVESTIGATOR'S NAME(S):		C. Krueger, S. K. Narang, M. Degrugillier and L. Heilmann
AFFILIATION & LOCATION:		USDA, ARS, BRL, Fargo, ND
ACTION AREA:	6.	Genetics, Molecular Biology and Basic Physiology
LEAD ARRAY:	6.1	Mechanism of Backcross Sterility in *Heliothis virescens*
SAFEGD ARRAY:	6.1.1	Genetic relatedness of RLOs and VLPs of *H. Zea, H. virescens,* and *H. subflexa*
DATES COVERED BY REPORT:		September 1991-April 1993

PROGRESS REPORT: Polymerase chain reaction and DNA sequence studies were undertaken for taxonomic identification of endosymbionts from the testes of *Heliothis virescens, H. subflexa* and backcross sterile (BCS) males. Earlier electron microscopic studies on testes had revealed the presence of rickettsia-like endosymbionts. The 16S rRNA gene of the endosymbionts was amplified from extracts of testes using appropriate bacterial primers. Electrophoresis of amplified products showed that the 16S rRNA genes of the endosymbionts of *H. subflexa* and BCS males were identical in size and were about 100 base pairs larger than the 16S rRNA gene (about 1.5 Kb) of endosymbiont of *H. virescens*. This supports our hypothesis that the endosymbionts in BCS males are derived from the female parent, *H. subflexa*, in the parental cross.

The DNA sequence of the 1.5 Kb amplified product was compared to the sequences of 16S rRNA genes in the GENE BANK data base, using FASTA search of the DNASIS and MACVECTOR programs. The sequence alignment data showed that the endosymbiont in *H. virescens* is a bacteria with close homology to *Agrobacterium tumefaciens, Rochalimaea quintana* and *Bartonella bacilliformis*. We are conducting PAUP analysis to determine the taxonomic identification and phylogeny of these endosymbionts. DNA sequencing work on 16S rRNA genes of endosymbionts from *subflexa* and BCS males is in progress for taxonomic identifications.

FY94 & FY95 WORK PLANS: The above research was conducted with the help of a Headquarters-funded research associate (Dr. Krueger). If we are successful in obtaining funds for a research associate position for the FY 94 and 95, we will pursue this project to determine the possible role of these endosymbionts in reproductive processes of the moth species. The approach would be to isolate aposymbiont strains of *Heliothis* by treatment with endosymbiont-specific antibiotics. In-situ hybridization studies will be used to determine relationships between the genomes of the endosymbiont and the moth. The results of these studies should provide foundation for validating the possible role of endosymbionts in backcross male sterility and for developing techniques for transferring sterility to *zea*.

INVESTIGATOR'S NAME(S): M. E. Degrugillier

AFFILIATION & LOCATION: USDA, ARS, BRL, Fargo, ND

ACTION AREA: 6. Genetics, Molecular Biology, and Basic Physiology

LEAD ARRAY: 6.1 Mechanisms of backcross sterility (BCS) in *Heliothis virescens* and transfer of BCS to *Helicoverpa zea*

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: Rickettsia-like organisms (RLOs) were found in the testes lumen of three groups of F1 hybrid males resulting from interspecific crosses between *H. subflexa* and *H. virescens*. These crosses involved *H. sub.* females X *H. vir.* males, the reciprocal of this cross, and a *H. sub.* female X *H. vir.* male cross in which the parental moths were captured from the wild. In addition, RLOs were found in BC1 and BC2 males resulting from the *H. subflexa* female X *H. virescens* male cross. Typical RLO intermediate bodies were not found in males of either *H.subflexa* or *H.virescens*, but putative RLOs were found associated with degenerating sperm of older males of both species. Putative RLOs were also commonly found in the cytoplasm of developing F1 spermatids. RLOs were not found in the testes of late generation backcross males (with one exception) but putative RLOs were commonly associated with the mitochondrial derivative of degenerating sperm. RLOs were also found in the testes of wild-caught *H. zea* (Arkansas).

INVESTIGATOR'S NAME(S):		R. L. Roehrdanz
AFFILIATION & LOCATION:		USDA, ARS, BRL, Fargo, ND
ACTION AREA:	6.	Genetics, Molecular Biology, and Basic Physiology
LEAD ARRAY:	6.2	Evaluate BCS as a control concept for *H. virescens* in the Mississippi Delta area
SAFEGD ARRAY:	6.2.1	Improve mass rearing and population monitoring technology for H. virescens
DATES COVERED BY REPORT:		October 1992-June 1993

PROGRESS REPORT: The release of sterile backcross males from [*(H. subflexa* f. X *H. virescens* m) females X *H. virescens* males] can be used to help reduce populations of *H. virescens*. A simple method is needed to measure the persistence or dispersal of the backcross sterility genetic factors. Backcross male sterility is transmitted via maternal inheritance. The mitochondrial DNA is also maternally inherited and thus "linked" to backcross sterility. The mtDNA in the backcross is from *H. subflexa*. Since *H. subflexa* is not a crop pest, the presence of its mtDNA among individuals collected from crops is an indicator of the presence of Backcross sterility factors. Several regions of the mtDNA were amplified using PCR, then cut with restriction enzymes. The restriction fragments were run on agarose gels and detected with ethidium bromide. Amplification can be obtained from single eggs, 1st or 2nd instar larvae, through adults. Species-specific pattern differences between *H. virescens* and *H. subflexa* (i.e. BC) were observed for the 16S, 12S-16S and COI-COII regions. The smaller 16S region can also be amplified from alcohol preserved larvae, which could eliminate the need to keep field collected material alive or frozen. The advantages of this technique are that it is quick and easy. A large number of individuals could be sampled in a short time period. A very small amount of DNA is sufficient and there is no need for hybridization, labelled probes, or autoradiography.

The same procedure can be used to distinguish *H. zea* from *H. virescens*. On crops where both species are found, determination of the species causing damage could be made at the egg or larval stage. There are a number of restriction pattern differences between these two species.

The data can also be used to define relationships among the Heliothinae species. The fraction of shared restriction fragments can be used as an indicator of genetic relatedness. Thus *H.zea* and *H. virescens* have 55% of the fragments in common, *H. zea* and *H. subflexa* have 56% in common, and *H. subflexa* and *H. virescens* 70% in common. This technique could help find the closest genetic relative of HZ among the world's species.

FY94 & FY95 WORK PLANS: Examine the possibility that purified RAPD-PCR fragments or cloned repetitive genomic fragments would demonstrate species specificity and permit development of a spot hybridization species identification assay.

INVESTIGATOR'S NAME(S):		J. DeVault and S. K. Narang
AFFILIATION & LOCATION:		USDA, ARS, BRL, Fargo, ND
ACTION AREA:	6	Genetics, Molecular Biology, and Basic Physiology
LEAD ARRAY:	6.2	Evaluate BCS as a control concept for *H. virescens* in the Mississippi Delta Area
OPTIM ARRAY:	6.2.2	Develop a genetic sexing system for *H. virescens* for production of BC progeny
DATES COVERED BY REPORT:		September 1991-April 1993

PROGRESS REPORT: As a first step to develop a gene transfer system, including genetic sexing, molecular studies were conducted to isolate transposable elements in *Heliothis* spp. Polymerase chain reaction experiments were conducted to amplify a hobo-like sequence in *virescens* and a mariner-like sequence in *H. zea* using degenerate primer sequences derived from corresponding *Drosophila* genes. A 373 bp DNA fragment amplified with hobo primer was cloned into the pGEM-T cloning vector. The DNA sequence of this fragment was determined. This clone was then used to probe a *virescens* lambda library to isolate genomic clones. A clone containing about a 9 Kb insert is being analyzed by restriction mapping and DNA sequencing to determine its potential as a transposon for gene transfer research. The DNA sequence of the amplified fragment showed approximately 51% identical DNA sequence homology with the hobo element and about 48% with P element of *Drosophila melanogaster*, as well as significant homology with mariner-like element of *C. elegans*. In addition, when this DNA was translated into protein, a significant amount of functional amino acid homology was observed between the PCR-generated clone and the transposase protein from Hobo.

The approximately 500 bp mariner-like fragment amplified from *zea* is present in some strains and absent in others, thereby suggesting that this element is functional and actively transposing. The DNA sequence work is in progress. Genomic clones of this element have also been isolated for characterization of complete mariner-like element.

FY94 & FY95 WORK PLANS: The plan for FY 94 involves cloning of full-size transposable elements, determining their sequences and testing their potentials as gene delivery vectors. The research plan for FY 95 will depend on the results of the above studies. If one or both of the above sequences show potential to be transposable elements, research plans will be developed to construct gene transfer vector system involving developmentally regulated and sex-specific genes. This work is being conducted with the support of a temporary research scientist (Dr. DeVault). Therefore, implementation of any research plans beyond FY 94 will depend on the extension of his current 2-yr appointment.

INVESTIGATOR'S NAME(S):		M. L. Laster[a], D. D. Hardee[a], and J. C. Schneider[b]
AFFILIATION & LOCATION:		[a]USDA, ARS, SFCIML, Stoneville, MS; [b]Department of Entomology, Mississippi State University, Mississippi State, MS
ACTION AREA:	6	Genetics, Molecular Biology, and Basic Physiology
LEAD ARRAY:	6.2	Evaluate BCS as a control concept for *H. virescens* in the Mississippi Delta area
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: A pilot test to evaluate the feasibility of controlling *Heliothis virescens* by mass rearing and releasing sterile backcross (BC) insects into the feral population was initiated in 1992. An average of 69,000 moths was released per day from an average of 81,500 pupae per day, for 43 days during the overwintered *H. virescens* emergence. Trap captures during the release period showed a 3.0:1.0 BC:wild ratio. This ratio dropped to 1.3:1.0 and 1.0:1.3 for June and July respectively. No suppression in the July *H. virescens* in the release area as a result of the release was identified.

Beginning 12 April 1993 an average of 68,000 moths per day was released from 80,000 pupae per day for 42 days. During the period of 4 April to 13 June, 4,993 BC and 6,942 wild moths were trapped in the release area. This resulted in a 1.0:1.4 BC:wild ratio for the release period.

The 1992 release area was monitored with pheromone traps to determine the size of the overwintered population and the incidence of male sterility from the 1992 release. During the period of 29 April to 21 June, 6,714 feral males were captured with 27.4 percent sterility. Progress is in year 2 of the 5 year plan.

FY94 & FY95 WORK PLANS: Evaluation of the pilot project will be completed. Both the 1993 release and control areas will be monitored in 1994 with pheromone traps to determine the incidence of male sterility in the feral populations resulting from the pilot release.

INVESTIGATOR'S NAME(S):		R. W. Poole
AFFILIATION & LOCATION:		Systematic Entomology Laboratory, National Museum of Natural History, Washington, DC
ACTION AREA:	6.	Genetics, Molecular Biology, and Basic Physiology
LEAD ARRAY:	6.3	Crossbreeding of *Helicoverpa* spp. to develop BCS in *H. zea*
SAFEGD ARRAY:	6.3.1	Obtain biosystematic and hybridization information on *Helicoverpa* species for future reference
DATES COVERED BY REPORT:		September 1991-June 1993

PROGRESS REPORT: Robert W. Poole of the Systematic Entomology Laboratory, ARS and Charles Mitter of the University of Maryland conducted research on the biosystematics of the Heliothinae under a USDA cooperative research grant. During this period a revision of the species of the *Heliothis virescens* species group was published. Thirteen species were recognized and 8 of them were described as new. A phylogenetic analysis of the species group was performed. A morphometric analysis of the populations of the tobacco budworm, *Heliothis virescens* was conducted. The morphometric analysis recognized three major subdivisions of the tobacco budworm. Of these three, the populations from the lesser Antilles is the most distinctive.

A phylogenetic analysis of the genus *Helicoverpa* was conducted using a combination of morphological and allo-enzyme data. This research is awaiting publication. The genus is divided into species group and the evolutionary relationships between the species detailed. The most interesting detail arising from the analysis is the very close relationship between *Helicoverpa zea* and *H. armigera* relative to other species in the genus.

FY94 & FY95 WORK PLANS: Current research with C. Mitter and Jerome Regier of the University of Maryland under a new USDA Cooperative Research Grant is a evolutionary analysis of the Heliothinae and their place in the Noctuidae as a whole. This analysis is being done with DNA sequencing techniques. This research is just beginning and no results are available.

INVESTIGATOR'S NAME(S): M. L. Laster

AFFILIATION & LOCATION: USDA, ARS, SFCIML, Stoneville, MS

ACTION AREA: 6 Genetics, Molecular Biology, and Basic Physiology

LEAD ARRAY: 6.3 Crossbreeding of *Helicoverpa* spp. to develop BCS in *H. zea*

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: A total of 19 *H. armigera* (A) collected from cotton in the former Union of Soviet Socialist Republics near Tashkent was used to study hybridization responses with *H. zea* (Z). Interspecific crosses, inbred crosses through the F_2 generation, and reciprocal backcrosses through five generations were carried out to determine mating incidence and fertility. Two pairs of A X Z mated and produced larvae. Mating incidence for the BC, (AZ X Z) was very low with only 5.3 percent of 38 pairs mating. The percent mating showed a general increase from the BC_2 through BC_5 with a high of 36.4 percent for BC_4 (AZ_3 X Z). The average number of eggs for reproductive females was high for all crosses but was lowest for $BC_{1\text{-}2}$ and their reciprocals. The percent egg hatch increased with each successive BC to a maximum of 86.4 percent for BC_5. No heritable backcross sterility was detected. Progress is in year 2 of the 5 year plan.

FY94 & FY95 WORK PLANS: The greatest problem is obtaining foreign material for crossing studies. We are scheduled to receive *H. armigera* from China in October or November of 1993.

INVESTIGATOR'S NAME(S):		J. E. Carpenter, H. R. Gross, and B. R. Wiseman
AFFILIATION & LOCATION:		USDA, ARS, IBPMRL, Tifton, GA
ACTION AREA:	6	Genetics, Molecular Biology, and Basic Physiology
LEAD ARRAY:	6.4	Potential use of inherited sterility as a control strategy for *H. zea*
SAFEGD ARRAY:	6.4.1	Effects of inherited sterility on *H. zea* physiology, behavior, and reproduction
DATES COVERED BY REPORT:		September 1991-June 1993.

PROGRESS REPORT: Irradiated (100 Gy), laboratory-reared *H. zea* moths were competitive with nonirradiated, laboratory-reared moths in attracting and securing mates under field conditions. Cytological examination of testes from progeny of irradiated males revealed that the percentage of F_1 and F_2 with visible chromosomal aberrations was dependent upon the dose of radiation administered to the P_1 male. Results from a study on the sperm-use patterns of *H. zea* indicated that sperm from irradiated males were competitive with sperm from normal males when the intermating interval of the female was 48h, but sperm competitiveness of the irradiated male was reduced when the intermating interval was 24h.

A pilot test was conducted in small mountain valleys in N.C. to assess the influence of released, substerilized (100 Gy) males on wild *H. zea* populations, and to measure the infusion rate of inherited sterility into the wild population. Results from this study revealed that the number of wild males captured per hectare was positively correlated with the distance from the release site of irradiated males. Analyses of seasonal population curves of wild *H. zea* males calculated from mark-recapture data suggested that seasonal increases of wild *H. zea* males were delayed and/or reduced in mountain valleys where irradiated males were released. The incidence of larvae with chromosomal aberrations (progeny of irradiated, released *H. zea* males collected from the test sites during the growing seasons) indicated that irradiated males were competitive in mating with wild females and were successful in producing F_1 progeny, which further reduced the wild population.

We investigated the effects of inherited sterility and host plant resistance on *H. zea* development, and found that larvae resulting from irradiated male by nonirradiated female crosses were equally competitive with normal larvae for all measured parameters. Additional laboratory and field studies have been initiated to measure the compatibility of inherited sterility and augmented natural enemies such as parasitoids and viruses. Preliminary data indicate that parasitoids and viruses are compatible with the inherited sterility method.

FY94 & FY95 WORK PLANS: Studies on the compatibility of inherited sterility and other control strategies will be continued. Emphasis will be placed on the integration of inherited sterility and parasitoids. Control strategies that demonstrate the greatest potential will be combined with inherited sterility and evaluated in preliminary field trials.

INVESTIGATOR'S NAME(S): A. C. Bartlett and N. J. Gawel

AFFILIATION & LOCATION: USDA, ARS, WCRL, Phoenix, AZ

ACTION AREA: 6. Genetics, Molecular Biology, and Basic Physiology

LEAD ARRAY: 6.5. Establish linkage groups and genetic maps of morphological mutants, allozymes, and DNA sequences

DATES COVERED BY REPORT: September 1991-June 1993

PROGRESS REPORT: A small number of individual adult specimens of female *Heliothis virescens*, male *Heliothis subflexa*, and the F1 progeny of the cross between these two species were obtained from Dr. Marion Laster. These specimens were analyzed by the RAPD-PCR (Randomly Amplified Polymorphic DNA - Polymerase Chain Reaction) technique for the detection and characterization of typically hybrid banding patterns. The individuals within each population showed clear polymorphic banding patterns which were clearly different between each species. A few of the bands in the F1 individuals could have been hybrid type bands. Unfortunately, we do not know whether the F1 progeny that we analyzed were progeny of the P1 individuals that we analyzed, thus we cannot clearly characterize them as hybrid patterns.

We have recently made single pair crosses of hybrid females from the *Heliothis* rearing facility at Mississippi State, Mississippi with *H. virescens* males from the Western Cotton Research Laboratory rearing facility and are now obtaining F1 progeny from each cross. We will run RAPD analyses of the parents and progeny to see if hybrid banding patterns can be detected in these crosses. This data should be available for presentation at the workshop.

FY94 & FY95 WORK PLANS: We need to acquire specimens of *H. subflexa* in order to make single pair matings with *H. virescens* to produce F1 and F2 progenies for analysis along with their respective parents. The identification of hybrid banding patterns is extremely important for further studies of the genetics of these sterile hybrids as well as for studies of hybrid matings in whitefly populations. If specimens are acquired from co-operators as requested, we will also conduct RAPD-PCR analysis of *H. virescens* for the development of geographical fingerprints in preparation for the identification of origin of individual moths.

INVESTIGATOR'S NAME(S): R. L. Roehrdanz

AFFILIATION & LOCATION: USDA, ARS, BRL, Fargo, ND

ACTION AREA: 6. Genetics, Molecular Biology, and Basic Physiology

LEAD ARRAY: 6.5 Establish linkage groups and genetic maps of morphological mutants, allozymes, and DNA sequences

DATES COVERED BY REPORT: October 1992-June 1993

PROGRESS REPORT: Mitochondrial DNA restriction site variability was surveyed in geographically divergent collections of *Heliothis virescens*. Individuals from four locations were used (Georgia, California, and two in Mexico). Two types of mtdna analysis were employed. The standard technique included digesting total DNA with restriction enzymes and hybridizing Southern blots with a labelled purified mtdna. Most of the same individuals were also tested for restriction site variation in two PCR-amplified portions of the mt genome, the 16S rDNA and the COI-COII regions. This latter approach was also used to test a smaller number of individuals from Tennessee, Mississippi, Oklahoma, and Weslaco (Texas), along with samples collected in April, June, July, and September in College Station (Texas).

For the total mtdna, 11 restriction enzymes were used that produced about 65 sites. These sites sampled about 350 base pairs of DNA out of the 16,000 base pair total mtdna. About 70 individuals from four locations were used (Tifton, Georgia; Brawley, California; Ciudad Obregon, Mex.; Hermosillo, Mex.). A restriction site map was also prepared for some of these sites. Most of the same individuals along with some additional insects were used for the PCR-based analysis. Seven restriction enzymes produced about 30 restriction sites and sampled about 175 base pairs of sequence.

Seven haplotypes were identified for the Southern blot data. Haplotype 1 (total) comprises 50/59 (85%) individuals. The other six haplotypes were found only once or twice. The PCR data also uncovered seven haplotypes. Haplotype 1 (PCR) was found in 118/131 (91%) individuals. The second most frequent haplotype comprised only 5% of the individuals. The other five haplotypes were found only once. Population distributions show that the rare haplotypes are scattered among the geographical locations.

MtDNA variability in *H. virescens* collections from diverse geographic locations is insufficient to warrant classification of the geographic collections as distinctive subpopulations. This would suggest that the overall population annually expands from a common reservoir population or that incoming migrants breed freely with those that may have overwintered locally. The findings are consistent with the reported migratory nature of the species, distribution of morphometric types in the western hemisphere, and a survey of enzymatic polymorphisms in the southern USA. No obvious seasonal differences were observed in the small number of individuals examined.

The results obtained from hybridization to total mtDNA and PCR amplification of selected regions lead to the same conclusion. Standard RFLP techniques have the drawback of being time consuming and limited by the amount of DNA that can be obtained from small individual insects. Complete sequencing of PCR amplified regions of mtDNA provides a wealth of information, but is both time consuming and expensive. Combining PCR amplification with RFLP analysis makes it possible to obtain data quickly from small samples of DNA and does not involve the use of radioactive isotopes.

A modified primer located in the COI gene was developed from an identified primer and published sequence data of other insects that greatly improves amplification of a 1700 base pair fragment spanning the COI and COII genes in *Heliothis* and a wide variety of other insects.

INVESTIGATOR'S NAME(S):		D. R. Nelson and J. S. Buckner
AFFILIATION & LOCATION:		USDA, ARS, BRL, Fargo, ND
ACTION AREA:	6	Genetics, Molecular Biology, and Basic Physiology
LEAD ARRAY:	6.6	Identify the physiological and biochemical basis of insect development, diapause, and reproduction as a component of area wide management
SAFEGD ARRAY:	6.6.1	Identify fundamental biochemical processes in insects
DATES COVERED BY REPORT:		March 1992-June 1993

PROGRESS REPORT: Laboratory-reared *H. virescens* and *H. zea* larvae, 2nd and 3rd instar, can be identified by gas chromatographic analysis of their surface lipids. Colonies were reared on the same diet and several generations sampled. Individual larvae are soaked in 200 ml of hexane for 5 min, the larva removed, and the hexane evaporated with nitrogen gas. The residue is redissolved in 100 ml of chloroform.

Analysis is performed on the sample by gas chromatography or gas chromatography-mass spectrometry. One ml of the chloroform solution is injected into a cool on-column injector connected to a 1 m retention gap connected to an 11 m capillary column of cross-linked dimethylsilicone. The column is temperature programmed from 150°C to 320°C at 4°C/min and held at maximum temperature for 5 min.

The larvae can be distinguished in 3 ways from the CGC-MS data: (1) the profile of the gas chromatographic traces are clearly different, (2) an alkene, hentriacontene, is a major component in *H. zea* and is not present in *H. virescens*, and (3) the methyl branch positions of the dimethylalkanes differ between the two species.

Colonized insects are often reared on artificial diet to which sorbic acid (2,4-hexadienoic acid) has been added to inhibit mold growth. When *Manduca sexta, H. zea* and *H. virescens* were reared on a standard wheat germ based diet, extra components were observed, by both thin-layer and gas chromatography, in surface lipids obtained from pupae of *H. virescens* when the diet contained low levels of sorbic acid. No significant amounts of extra components were observed in surface lipids from pupae of *M. sexta* and *H. zea* unless 2- to 3-fold higher amounts of sorbic acid were added to the diet.

The new lipid components were characterized as wax esters, the major alcohol being C26 (hexacosanol), with the acid moiety being either sorbic acid or partially reduced forms of sorbic acid. It appears that *H. virescens*, and perhaps *H. zea* have a very active esterifying enzyme system capable of utilizing available organic acids.

Components of the cuticular lipids serve as semiochemicals in a large number of insects. They may affect aggregation, mating behavior, and predation. Thus, in mass-rearing situations, or in rearing insects for behavioral studies, a possible affect of diet additives on insect behavior must be kept in mind.

TABLE 6. Summary of Research Progress for Action Area VI, Genetics, Molecular Biology, and Basic Physiology, in Relation to Year 2 Goals of the 5-Year Plan.

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
6.1 Mechanisms of backcross sterility in *Heliothis virescens* and transfer of BCS to *Helicoverpa zea*.	Continue taxonomic identification and molecular, biochemical, and ultrastructure characterization of Rickettsia-Like Organisms.	X		Electrophoresis showed the 16sRNA genes of the endosymbionts of H. subflexa and BCS males were identical supporting the hypothesis that the BCS endosymbionts are derived from the female *H. subflexa parent*. Sequence alignment data showed the endosymbiont of *H. virescens* had close homology to *Agrobacterium tumefaciens*, *Rochalimaea quintana* and *Bartonella bacilliformis*. Rickettsia-like organisms were found in the testes of three groups of F_1 hybrid males. RLO's were also found in BC1 and BC2 males. Putative RLO's were found with degenerating sperm of older males of both *H. subflexa* and *H. virescens* and in the cytoplasm of developing F_1 spermatids. No progress was reported in arrays 6.1.2 or 6.1.3.
6.2 Evaluate BCS as a control concept for *H. virescens* in the Mississippi Delta.	Release BC males and females over a 9 X 9 mi area in the spring. Incorporate quality control measures into mass rearing technology.	X		An average of almost 70,000 moths were released daily during the *H. virescens* overwintering emergence period during 1992 and 1993. In 1992,trap captures indicated a 3:1 BC:wild initial ratio which dropped to 1:1.4 in July. Male sterility in May and July of 1992 averaged 27.4%. Methods were devised to monitor the presence of BC using mtDNA from any stage of field collected specimens. A very small amount of DNA is required to perform the test. The procedure can be used to distinguish *H. zea* from *H. virescens* and the genetic relatedness of species. Research is progressing to isolate transposable elements in *Heliothis* spp. for use in developing a gene transfer system which will include genetic sexing. No progress was reported on array 6.2.1.

TABLE 6 - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
6.3 Crossbreeding of *Helicoverpa* spp. to develop BCS in *H. zea*.	Continue collections of *Helicoverpa* spp. and crossbreeding experiments.	X		*H. armigera* from Tashkent crossed with H. zea resulted in progeny from 2 pairs. Initial mating between the BC female and *H.zea* males was only 5.3%, however, this increased to 36% for BC_4 crosses. No heritable backcross sterility was detected. A phylogenetic analysis of the genus *Helicoverpa* was conducted using morphological and alloenzyme data. *H. zea* and *H. armigera* exhibited very close relationships.
6.4 Potential use of inherited sterility as a control strategy for *H. zea*.	Continue studies on the interaction of inherited sterility and *H. zea* behavior and physiology, and initiate studies to examine the compatibility of inherited sterility in *H. zea* with other control strategies.	X		Irradiated *H. zea* moths were competitive in securing mates under field conditions. Visible percentage of chromosomal aberrations resulting from substerile irradiation was dependant on dose. Release of substerile males resulted in infusion of inherited sterility into a wild population. Data suggested the released males were competitive with the wild and a population reduction was observed. Preliminary data indicate that parasitiods and viruses are compatible with the inherited sterility technique. No progress was reported on arrays 6.4.2 or 6.4.3.
6.5 establish linkage groups and genetic maps of morphological mutants, allozymes, and DNA sequences.	Continue crosses to map genes, complete construction of genomic library, isolate clones of known sequences.	X		RAPD-PCR analysis of *H. virescens* and *H. subflexa* showed different polymorphic banding patterns. F_1 progeny exhibited possible hybrid band types. MtDNA variability in H. virescens collections from diverse geographic locations was insufficient to warrant classification of the collections as distinctive subpopulations. This suggests the overall population annually expands from a common reservoir or that incoming migrants breed freely with local populations. Results obtained from hybridization to total mtDNA and PCR amplification of selected regions led to the same conclusion. No progress was reported on arrays 6.5.1 or 6.5.2.

TABLE 6 - Continued

ARRAY	YEAR 2 GOALS	PROGRESS YES	PROGRESS NO	SIGNIFICANCE
6.6 Identify the physiological and biochemical basis of insect development, diapause, and reproduction as a component of area wide management.	Purify and characterize urate storage in H. virescens pupal fat body; compare composition of surface lipids in other lepidoptera and the occurrence of internal alcohols and esters.	X		Lab reared *H. virescens* and *H. zea* were identified by analysis of their surface lipids. When *Manduca sexta*, *H. zea* and *H. virescens* were reared on a standard wheat germ diet extra components were observed in surface lipids obtained from *H. virescens* pupae, however no significant amounts of extra components were observed in surface lipids from *M. sexta* or *H. zea* unless 2- to 3-fold higher amounts of sorbic acid were added to the diet. These findings may have significance in using lab reared insects for behavioral studies. No progress was reported for arrays 6.6.1 or 6.6.2. No activity was scheduled for 6.6.3.

RESEARCH SUMMARY: ACTION AREA VI—GENETICS, MOLECULAR BIOLOGY, AND BASIC PHYSIOLOGY

Compiled by J. E. Carpenter and A. C. Bartlett

The *Heliothis/Helicoverpa* working conference report and National Action Plan of 1991 identified six areas in which insect genetics, molecular biology, and basic physiology could provide major contributions to the discovery, development, and refinement of alternative management approaches for *Heliothis/Helicoverpa* species. These six areas are: (1) the elucidation of the mechanism responsible for backcross sterility in *H. virescens* and the transfer of backcross sterility to *H. zea*, (2) the evaluation of backcross sterility as a control concept for *H. virescens* in the Mississippi delta area, (3) the crossbreeding of *Helicoverpa* spp. to develop backcross sterility in *H. zea*, (4) the potential use of inherited sterility as a control strategy for *H. zea* and *H. virescens*, (5) the development of genetic sexing systems for *H. zea* and *H. virescens* to eliminate the production of females, and (6) elucidation of physiological and biochemical bases of development, diapause, and reproduction in *Heliothis/Helicoverpa*.

LEAD ARRAY 6.1: Studies on taxonomic identification and molecular (DNA), biochemical and ultrastructure characterization of Rickettsia-Like-Organisms (RLO's) support the hypothesis that certain endosymbionts associated with the testes in backcross males are derived from the original female parent (*H. subflexa*). Good progress has been made in the identification of RLO's except in *H. zea*, however, no progress has been reported on the molecular biology of virus-like particles (VLP) or on the development of aposymbiont strains of *Heliothis* spp.

LEAD ARRAY 6.2: Sterile backcross insects were released in the Mississippi delta during the growing seasons of 1992 and 1993. In the spring of 1993, 27.4% of males captured in this area were sterile hybrids. Restriction fragments of mitochondrial DNA from *H. virescens, H. subflexa,* and *Heliothis* hybrids were diagnostic for species identification in many developmental stages of the insects. Studies on germ-line transfer technology found potential transposable element fragments in *H. virescens* and *H. zea*. No progress has been reported on the isolation or characterization of sex-linked, conditional lethal mutations for the development of a genetic sexing system in *H. virescens*.

LEAD ARRAY 6.3: Limited hybrization attempts between *H. armigera* and *H. zea* showed no apparent heritable backcross sterility. A phylogenetic analysis of the genus *Helicoverpa* conducted using a combination of morphological and alloenzyme data demonstrated a very close relationship between *H. zea* and *H. armigera* relative to other species in the genus. Crossbreeding experiments of *Helicoverpa* spp. to develop backcross sterility in *H. zea* are on-going, however test crosses have been of limited scope due to the difficulty of obtaining adequate sample sizes and diversity.

LEAD ARRAY 6.4: In a pilot test, inherited sterility demonstrated that seasonal increases of wild *H. zea* males were delayed and/or reduced in areas where partially sterile males were released. Sperm from partially sterile males were competitive with sperm from untreated males. Studies indicate that the inherited sterility method is compatible with host plant resistance and other biological control strategies. No progress has been made to develop a genetic sexing system for *H. zea* to eliminate the production of females. No work has been done to study the response of *H. virescens* to substerilizing doses of radiation.

LEAD ARRAY 6.5: Studies of mitochondrial DNA restriction site variability throughout several geographical locations identified seven haplotypes of *H. virescens*. A restriction site map was prepared for some of the locations. These data are consistent with the hypotheses that the overall population expands annually from a common reservoir population or that incoming migrants breed freely with those that overwintered locally. A limited RAPD-PCR analysis of *H. virescens, H. subflexa,* and their F_1 hybrids showed that banding

polymorphisms were present in these populations, but the bands from the F_1 progeny could not be characterized as hybrid patterns. Good progress has been made in construction of a genomic library for the mitochondrial DNA of *H. virescens*, however no progress has been reported on the establishment of genetic mutant stocks, linkage maps, or extensive surveys of allozyme variation. As a result no progress has been made on characterization of gene expression during the life cycle of the insect species.

LEAD ARRAY 6.6: Laboratory reared *H. virescens* and *H. zea* larvae can be distinguished by the profile of gas chromatographic traces of their surface lipids, the presence of an alkene, hentriacontene in *H. zea* surface lipids, and by differences in the methyl branch positions of the dimethylalkanes. *H. virescens* (and perhaps *H. zea)* has an active esterifying enzyme system which may be capable of utilizing organic acids present in the diet to produce newly identified lipid components. No progress was reported on the function of uric acid or internal and cuticular lipids, or on the timing of uric acid storage in pupal fat body and subsequent release in the adult.

The progress made in the area of genetics, molecular biology, and basic physiology should provide major contributions to the discovery, development, and refinement of alternative management approaches for *Heliothis/Helicoverpa* species. Of the seven research gaps identified in the *Heliothis/Helicoverpa* National Action Plan, four items are currently under investigation and are showing promising results. For example, the recent identification of portions of transposable elements could rapidly accelerate gene transfer technology for these pest species. This technology has significant implications for numerous areas of research, such as the development of a genetic sexing system. An automated genetic sexing system would increase the effectiveness of any autocidal suppression program, such as inherited sterility and backcross sterility.

BREAKOUT SESSION SUMMARY

The *Heliothis/Helicoverpa* working conference report and National Action Plan of 1991 identified six areas in which insect genetics, molecular biology, and basic physiology could provide major contributions to the discovery, development, and refinement of alternative management approaches for *Heliothis/Helicoverpa* species. During the breakout session of the *Heliothis/Helicoverpa* Annual Review (Junction, TX, 1993), participants in Action Area 6 reported that research for the first two years of the Action Plan was currently on track for most of the research arrays. The work involving larval surface hydrocarbons (Array 6.6.1) was identified for redirection because the research goals of this project were satisfied with the development of an immunodot assay capable of differentiating *H. zea* and *H. virescens* eggs (Array 5.2.3c). No other changes were suggested for Action Area 6.

Appendix A. Publication List, 1991-1993

Akins, D.C., T.L. Wagner, J.L. Willers, et al. 1993. New graphical user interface for the rbWHIMS insect management expert system. Proc. Beltwide Cotton Prod. Res. Conf., Natl. Cotton Council, Memphis, TN. In press.

Archer, T.L., B.R. Wiseman, & A.J. Bockholt. 1993. Factors affecting corn earworm (Lepidoptera: Noctuidae) resistance in food corn. J. Agric. Entomol. 10. In press.

Beerwinkle, K.R., J.D. Lopez, Jr., & J.A. Witz. 1991. Temporal patterns of bollworm and tobacco budworm male captures in traps baited with virgin females and synthetic pheromones. Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN. (abstract).

Beerwinkle, K.R., J.D. Lopez, Jr., J.A. Witz, et al. 1991. Seasonal radar observations of upper-air nocturnal insect activity and related meteorology in East-Central Texas. *In* Proc. of Tenth Conf. on Biomet. and Aerobiol. Amer. Meteor. Soc., Boston.

Beerwinkle, K.R., J.A. Witz, & J.L. Goodenough. 1992. An improved electromechanical moth capture detection system for pheromone-baited cone traps. Appl. Engin. in Agric., ASAE. 8:113-118.

Beerwinkle, K.R., T.N. Shaver, & J.D. Lopez, Jr. 1993. Field observations of adult emergence and feeding behavior of *Helicoverpa zea* (Lepidoptera: Noctuidae) on dallisgrass ergot honeydew. Environ. Entomol. 22:554-558.

Beerwinkle, K.R., J.A. Witz, & P.G. Schleider. 1993. An automated vertical-looking x-band radar system for continuously monitoring aerial insect activity. Transactions of ASAE. (Accepted February 1993).

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Bell, M.R. 1990. A new way to control tobacco budworm and bollworm. Mississippi State University misc. publication No. M0532.

Bell, M.R. 1990. Use of baculovirus or the control of *Heliothis* spp. in area-wide pest management programs. Proc. Vth International Colloquium on Insect Pathology and Microbial Control. Adelaide, Australia, August 20-24, 1990, pp. 486-90.

Bell, M.R., D.D. Hardee, J.L. Hayes, & E.A. Stadelbacher. 1990. Management of cotton bollworm/tobacco budworm populations through area-wide application of nuclear polyhedrosis virus on early-season hosts. Proc. Joint Soviet/American Conference on Insect Pest Management. Tashkent, Soviet Union, September 1990.

Bell, M.R., & D.D. Hardee. 1990. Management of Bollworm/Budworm Populations Through Area Wide Application of Nuclear Polyhedrosis Virus. Proc. 37th Ann. Mississippi Insect Control Conference. Nov. 1990 Mississippi State, MS. (Abstract).

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Bell, M.R. 1991. Effectiveness of microbial control of *Heliothis* spp. developing on early season wild geraniums: Field and field cage tests. J. Econ. Entomol. 84:851-854.

Bell, M.R. 1991. *In vivo* production of a nuclear polyhedrosis virus utilizing tobacco budworm and a multicellular larval rearing container. J. Entomol. Sci. 26:69-75.

Bell, M.R. 1991. Mass *in vivo* production of an entomopathogenic virus for use in large scale tests. Proc. 38th Ann. Mississippi Insect Control Conference. Nov. 1991 Mississippi State, MS. (Abstract).

Bell, M.R., & D.D. Hardee. 1991. Aerial application of an entomopathogenic virus as an area wide management program of early season bollworms and budworms. Proc. XII International Plant Protection Congress. August 11-16. Rio de Janeiro. (Abstract).

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Appendix B. Meeting Agenda

Guest of Honor: E. F. Knipling

Monday, November 8, 1993 Arrive

5:00 - 6:00 PM:	Evening Meal
6:00 - 7:00 PM:	Steering Committee and Coordinators meeting

Tuesday, November 9, 1993

8:00 - 8:15 AM:	Welcome to Conference	F. P. Horn
8:15 - 8:30 AM:	Charge of Workshop Participants	J. L. Krysan
8:30 - 8:45 AM:	Industry Perspective	A. Jordan
8:45 - 9:00 AM:	CSRS-SAES Perspective	J. R. Cate
9:00 - 9:15 AM:	Consultant Perspective	S. Nemec
9:15 - 9:30 AM:	Grower Perspective	G. Long
9:30 - 9:45 AM:	Chemical Industry Perspective	D. Allemann
9:45 - 10:00 AM:	ARS National Program Perspective	R. Faust
10:00 - 10:30 AM:	Break	

SUMMARY REPORTS OF ACTION AREAS

		Coordinators
10:30 - 10:45 AM:	Host Plant Resistance	L. Lambert & B. D. Barry
10:45 - 11:00 AM:	Chemical Control	I. W. Kirk & D. A. Wolfenbarger
11:00 - 11:15 AM:	Ecology and Populations Dynamics	J. D. Lopez & T. W. Popham
11:15 - 11:30 AM:	Behavior Modifying Chemicals	A. K. Raina & T. N. Shaver
11:30 - 11:45 AM:	Biological Control	J. J. Hamm & G. Tillman
11:45 - 12:00 AM:	Genetics & Molecular Biology	J. E. Carpenter & A. C. Bartlett
12:00 - 1:00 PM:	Lunch	

	All Participants	**Session Leaders**
1:00 - 3:00 PM:	**Work Session:** Host Plant Resistance	
1:00 - 1:30 PM:	Discussion of Arrays	Coordinators
1:30 - 1:50 PM:	"Recent Developments in Bt Research in Transgenic Plants"	R. G. Luttrell
1:50 - 3:00 PM:	Facilitators: Hard Questions, Correction of Arrays, Etc.	J. R. Coppedge & H. R. Gross
3:00 - 3:30 PM:	Break	
3:30 - 5:50 PM:	**Work Session:** Chemical Control	
3:30 - 4:00 PM:	Discussion of Arrays	Coordinators
4:00 - 4:20 PM:	"Variables in Spray Deposits & Drift	L. F. Bouse
4:20 - 5:30 PM:	Facilitators: Hard Questions, Correction of Arrays, Etc.	J. R. Coppedge & E. R. Mitchell
7:00 - 8:00 PM:	Dinner; *Heliothis/Helicoverpa* Problem From a Broad Perspective	E. F. Knipling

Wednesday, November 10, 1993

	All Participants	Session Leaders
7:30 - 9:30 AM:	**Work Session:** Ecology, Population Dynamics	
7:30 - 8:00 AM:	Discussion of Arrays	Coordinators
8:00 - 8:20 AM:	"New Concepts in Migration Research	J. K. Westbrook
8:20 - 9:30 AM:	Facilitators: Hard Questions, Correction of Arrays, Etc.	J. R. Coppedge & T. Henneberry
9:30 - 10:00 AM:	Break	
10:00 - 12:00 AM:	**Work Session:** Behavior Mod. Cemicals	
10:00 - 10:30 AM:	Discussion of Arrays	Coordinators
10:30 - 10:50 AM:	"Recent Developments in Attracticide Research"	K. R. Beerwinkle
10:50 - 12:00 AM:	Facilitators: Hard Questions, Correction of Arrays, Etc.	J. R. Coppedge & P. D. Lingren
12:00 - 1:00 PM:	Lunch; ARS of the Future	F. P. Horn
1:00 - 3:00 PM:	**Work Session:** Biological Control	
1:00 - 1:30 PM:	Discussion of Arrays	Coordinators
1:30 - 1:50 PM:	"Trends in Research With Nematodes"	J. R. Raulston
1:50 - 3:00 PM:	Facilitators: Hard Questions, Correction of Arrays, Etc.	J. Krysen & D. D. Hardee
3:00 - 3:30 PM:	Break	
3:30 - 5:30 PM:	**Work Session:** Genetics, Molecular Biology, Basic Physiology	
3:30 - 4:00 PM:	Discussion of Arrays	Coordinators
4:00 - 4:20 PM:	"Genetic Variation in H/H Populations"	K. S. Narang
4:20 - 5:30 PM:	Facilitator: Hard Questions, Correction of Arrays, Etc.	R. M. Faust

Thursday, November 11, 1993

8:00 - 9:00 AM:	Reviewers Report
9:00 - 10:00 AM:	Breakout Sessions
10:00 - 10:30 AM:	Break
10:30 - 12:00 AM:	Steering Committee - Coordinators Meeting

Appendix C. List of Registered Participants

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Appendix D. Action Area Presentations

Keynote Presentation

A LOOK AT THE *HELIOTHIS/HELICOVERPA* PROBLEM FROM A BROAD PERSPECTIVE

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The Heliothis complex, *Helicoverpa zea* and *Heliothis virescens* is responsible for the Nations most costly insect pest problem. The losses the two pests cause are estimated to range up to 2 billion dollars per year. Much of the Nation's research effort on insects is focused on these two pests. The nature and extent of this effort are indicated by the wide range of topics for discussion on the conference agenda. I appreciate the opportunity to participate in the conference and wish to briefly analyze where we stand today in dealing with these pests and suggest what might be done to eliminate the large losses they cause under current management procedures.

The Current Status of *Heliothis* Management

In dealing with what I will call the *Heliothis* complex, we are confronted with species that attack a number of our major crops and a hundred or more minor crops and wild host plants. Each growing season populations spread from relatively low numbers in their somewhat restricted overwintering areas, and reach high density levels throughout most of the Nation. For many years, growers had to depend entirely on natural control factors and accepted the losses they cause. However, for the past half century, an number of chemical and biological insecticides have become available that growers can use to alleviate the damage. Plant breeders and entomologists have also developed varieties of corn and some other crops that have considerable resistance and tolerance to *Heliothis* attack. These developments have saved growers and our agriculture economy billions of dollars. But, despite intensive research efforts for 50 years, the annual losses these pests cause continue to be high; and judging from the estimates made from time to time, the losses seem to be increasing. Also, there is growing concerns over the insecticide resistance problem and the possibility that there will be more and more restrictions on the use of environmentally hazardous insecticides.

The Dynamics of Heliothis/Helicoverpa Populations and the Losses These Pests Cause Under Present Control Procedures

When the dynamics of *Heliothis* populations under current control procedures are analyzed from a broad perspective, we can readily understand why the losses due t the pest continue to remain high, and rather consistent from year to year. After effective insecticides became available, it was soon recognized that their broad spectrum activity seriously disrupted the action of natural biological control agents. Accordingly, the use of such insecticides was discouraged unless absolutely necessary. Thus, *Heliothis* management evolved as a defensive and reactive pest management procedure. Growers were urged to apply insecticide only when survey data indicated that losses would likely exceed the cost of control. This procedure served useful purposes. Individual growers profited by not making or delaying treatments until necessary. They also profited by avoiding excessive losses. This management procedure also limited the amount of environmentally hazardous insecticides released in our agro-ecosystems, one of the major objectives of integrated pest management.

However, virtually nothing was or could be done by individual growers, acting on their own, to prevent the normal early season build up and spread of *Heliothis* populations. Therefore, the overwintering populations can increase unhampered until they reach economic threshold levels on the various crops at risk. In general, however, populations must grow to rather high levels and be capable of causing considerable damage before the cost of control by insecticides will be profitable. *H. zea* on corn is a good example of the damage the pests can

cause under such circumstances. The damage caused by *H. Zea*, the corn earworm, probably averages near $15 per acre. However, it costs more than $15 per acre to prevent such loss. Other host crops have different loss/benefit equations, but the principle is the same. There is a large loss to agriculture year after year before the present control procedures become profitable. I have not seen data to support the estimate, but it is probable that 75 percent of the losses caused by the two pests normally fall below the present control costs. Thus, if the potential loss on all crops is $2 billion per year, growers and the agriculture economy can be expected to suffer an annual loss of about $1.5 billion, plus the cost of control.

This in essence is where we stand today in dealing with the *Heliothis/Helicoverpa* problem. If we analyze the influence of present control procedures on the seasonal dynamics of *Heliothis* populations, we can fully appreciate the reason why losses due to the pests remain high from year to year. It is probable that not more than 10 to 15 percent of the total populations will be killed by the methods of control employed. Also, when broad spectrum insecticides are applied, the increase rate of the survivors and their progeny is above normal. Therefore, the total number of the insects that is produced each season is likely to be about the same despite the control efforts. Thus, the overwintered populations will be near normal in size and cause the same risks to agriculture year after year.

While there may be some flaws in the estimates and assumption made, I believe that the foregoing realistically describes the limitations of present *Heliothis/Helicoverpa* management practices. Unfortunately, the present conditions can be expected to continue for the indefinite future unless new control techniques and strategies for their management are developed and put into practice.

The Total *Heliothis* Population Management Concept

It has long been my view that the most successful way to cope with *H. zea* and *H. virescens* (and an number of other major insect pests) would be stress prevention and attack the total populations in an organized and coordinated manner before the populations reach damage levels, rather than follow the prevailing defensive strategy of controlling the pests on a farm to farm and crop to crop basis as the need arises. Accordingly, I encouraged research on area-wide management procedures for some years. But, it seemed questionable if it would ever be feasible to deal in this manner with a pest complex that attacks hundreds of different kinds of host plants and that can migrate for a thousand miles or more during a single season. However, in view of the advances that *Heliothis* scientists have made during the past several decades on virtually every aspect of the problem that has relevance to the total insect population management concept, I no longer have such reservations.

Instead, it is now my conviction that despite the magnitude of such effort, rigid management of *Heliothis* populations could be achieved each year at costs that would be only a fraction of the losses they now cause. Also, that this could be accomplished without significant environmental hazards. Indeed, in view of the experience gained from almost 50 years of efforts to deal with these pests in the manner described, I have reached the firm conclusion that the total population area-wide management approach is our only real hope of coping with the pests in a highly effective, low cost, and ecologically sound manner.

While relatively little of the vast amount of new information obtained by *Heliothis* scientists during the past several decades has been put into practice, their research findings have been outstanding. Ecologists investigating the relationship of the insects to their environment have obtained a good understanding of the role of wild host plants in permitting the early season build up of populations. They have also confirmed what has long been known, that the insects have very long range dispersal capabilities. Biologists working on natural enemies of the pests have discovered new parasites, predators and microbial agents that attack the pests. Insect biologists working on the relationship of certain parasites and their *Heliothis* hosts have shown that host specific parasites have highly developed mechanisms for detecting the host habitats and host locations. This alone suggests that there is little or no positive correlation between host densities and the host finding efficiency of the

individual parasites. There is no question that the ratio of parasites to hosts in coexisting populations determines the proportion of the host population that will be parasitized, irrespective of the density of the host population. These basic findings have very important implications for making practical use of the parasite augmentation technique for suppressing and maintaining the pest populations below economic density levels.

Biochemists have identified and synthesized the chemical components that the insects use for sexual communication. This not only makes available highly sensitive methods of detecting where and how many of the insect exits in various habitats, the pheromones might be useful for controlling low density populations. Attractant components in host plants are under investigation. Insect geneticists and entomologists have discovered genetic mechanisms that inhibit reproduction much more effectively than the conventional sterility technique.

Of special significance, insect rearing specialists have made outstanding advances on the mass production of the various life stages of the two *Heliothis* species. This makes possible the mass production of various parasites, pathogens, and other biological organisms that might be needed for augmentation purposes, and the mass production of moths for autocidal control.

All of these developments and others, have relevance to the total population area-wide management concept. Unfortunately, however, most of the new information has remained largely stagnated for a number of years because the scientists have not had the resources to make the transition from basic to applied technology.

Total Population Suppression Strategy

Obviously, it would take much time and space to outline in detail how total population management can be achieved by various control techniques when used alone or when integrated. But, some general comments can be offered.

I envision that rigid management of *Heliothis* populations will involve two phases. The objective of the first phase, using all available technology, will be to reduce the normal high populations to a much lower level. This might involve the management of wild host plants, the release of large numbers of certain parasites, the release of genetically altered moths, the use of attractants, and the application of biological or chemical insecticides. The integration of two or more techniques is likely to be advantageous. The suppressive measures would be focused at appropriate times against the first and second generations in the principle overwintering areas. The cooperation and participation of our colleagues with the Republic of Mexico will be essential. Ecologists have found that early season populations developing in Mexico are one of the most important sources of migrant moths.

In the case of the corn earworm, there is no question that corn is the key host plant. The rigid management of the populations developing on about 7 million acres of corn in the principle overwintering areas in the U. S. and Mexico, might alone be adequate to achieve the objective during phase 1. Several techniques could be used to prevent reproduction on corn. This would not only avoid the damage the pests cause on corn, it would reduce the populations on the other host crops.

If the normal overwintering populations can be reduced by perhaps 75 percent during phase 1, further suppression should be possible during phase 2 with less effort and lower cost. In such event, the routine release of relatively few *Heliothis* specific parasites and/or genetically altered moths each year should maintain total populations below significant damage levels on all of the crops. I believe that the total cost even during phase 1 would not exceed 25 percent of the annual losses. But the real payoff would be during phase 2. In my opinion, *H. zea* and *H. virescens* populations could be maintained below significant damage levels on all crops at a cost that would not exceed $100 million per year. This would be only about 5-10 percent of the estimated annual losses under present management procedures and could be accomplished without hazards to the environment.

It will be a major challenge for scientists to perfect the technology needed and demonstrate its utility. It will be an equal challenge for pest managers to plan, organize, and execute a program of such magnitude. But, I see no biological or technical barriers that would preclude success of such effort.

My greatest concern will be to convince our agricultural leadership in both the public and private sectors, and our political leadership, to provide the substantial financial resources that will be required by scientists to deal with the pests in the manner proposed. However, if this is not done, it is virtually certain that the two *Heliothis* species will continue to cots growers and our agriculture economy more than a billion dollars each year for the indefinite future. And, the probability will be high that there will be a continuing need to rely heavily on ecologically disruptive insecticides for *Heliothis/Helicoverpa* control.

I would like to close by offering some other general comments. The transition from the control of the more important insect pests on a farm to farm basis as the need arises to the regulation of total populations on an area-wide basis, will require drastic changes in pest management procedures. Individual growers no doubt will have to finance much of the costs, but programs will have to be executed by organizations or agencies that have the resources and the expertise to apply the technology in an effective and efficient manner. If biological methods are employed, facilities for mass producing the organism needed will have to be provided and operated by responsible organizations. How to finance such programs in an equitable manner will be a matter that must be resolved. To deal with pests like the fall armyworm, cabbage looper and a number of other pests, the most logical procedure will be to suppress populations in the greatly restricted overwintering areas. Growers in the non-overwintering areas to which these pests spread each season might profit as much or more from such programs that the growers in the areas where control operations will be conducted. This is also true in part for the *Heliothis* complex. Some equitable procedures will have to be formulated to finance such programs.

While area-wide management poses a number of problems, the benefits fully justify the difficulties that are involved. The livestock industry in the U. S. and Mexico have benefitted by about $10 for every dollar spent in conducting the screwworm programs. I have said from time to time that we have or can develop the technology to rigidly manage total populations of a dozen or more of our other major insect pests with comparable benefits in relation to costs, if we will apply the technology we have or can have in a similar manner. And, this can be accomplished with little or no hazard to the environment.

Consultant Perspective Presentation

COTTON INSECTICIDE RESISTANCE AND MANAGEMENT IN TEXAS

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Three periods of insect resistance to cotton insecticides have occurred in the USA during the past three decades. To fully address the problem of insecticide resistance to pyrethroids in the 1990s, we need to review how we survived resistance by boll weevils and bollworms to chlorinated hydrocarbons in the mid-1960s and resistance by budworms to organophosphate in the early 1970s and to synthetic pyrethroids in the 1980s.
From the mid-1950s to the mid-1960s, the main cotton pests in the Brazos and Rio Grande Valleys of Texas were aphids, fleahoppers, boll weevils and bollworms. Control of these key pests was attained by a scheduled "washday" program. Cotton was sprayed on a seven-day interval from emergence to first bloom; then the schedule was reduced to five-day intervals until maturity.

The chemicals available during this time were all organoclorines - DDT, endrin, toxaphene, dieldrin, BHC, heptachlor. A mixture of toxaphene plus DDT evolved as the predominant chemical for cotton insect control. This mixture was highly effective, had a relatively long residue, and control of all of the insect pests was excellent. However, between 1963-1965 the toxaphene-DDT mixture began to fail in many areas due to the overuse of scheduled applications and increasing insect resistance.

Methyl to the Rescue

Methyl parathion, the first organophosphate for insect control was shown to be highly effective against the boll weevil and other cotton pests at very low rates. This pesticide, formulated with toxaphene and DDT became the main chemical used on cotton, still on a washday schedule. However, I began recommending toxaphene plus methyl parathion without DDT for bollworm boll, weevil control and tobacco budworm control on an as-needed basis rather than a washday program. In 1967, *Heliothis/Helicoverpa* infestations were very severe and most of the cotton in the Lower Rio Grande Valley was sprayed with ultra low volume methyl parathion, with almost 100 percent insect control resulting. By 1968, the ULV spray method was also being used in the Brazos Valley. Insect control on the farms I checked consisted of seven applications of methyl parathion, compared to the 10 to 16 applications of toxaphene plus methyl of previous years.

The Inevitable

Just as we thought we had our cotton insect problems under control, the inevitable happened. In 1968 in the Rio Grande Valley and 1969 in the Brazos Valley, tobacco budworms became difficult to control, even with high rates of ULV methyl parathion. Application at twice the recommended rate gave little or no control, and reducing the application interval to two or three days had little effect. Cotton yields were greatly reduced and several farmers went out of business. The tobacco budworm problem in both areas was twofold: the obvious development of resistance to the organochlorine and OP insecticides, and extremely high insect populations. It was very common to have 25 to 50 eggs per terminal nightly. Fifty eggs per terminal represents about 2 million eggs per acre--99 percent control still left 20,000 larvae per acre.

Breakthrough

A breakthrough occurred in 1971 when chlordimeform was shown to be highly effective as an ovicide. Tobacco budworm control was fairly effective from 1972 to 1979 when mixtures of toxaphene, methyl parathion and chlordimeform were applied during oviposition or immediately following larval eclosion. Field testing of the promising new synthetic pyrethroid chemistries began in 1976-78. By 1979, the EPA had granted emergency

registration of Ambush, Pounce, Pydrin, Bolstar and Lannate for control of tobacco budworm on cotton. Synthetic pyrethroids became the dominant pesticides for control of cotton pests. Initially, spray intervals of 10 days to 14 days provided excellent control of Heliothis spp. The pyrethroids alos provided effective effective control of bollworms at the lowest labeled dosages, and were used extensively throughout the cotton growing season for control of the *Heliothis/Helicoverpa* complex. However with the extended application intervals, the boll weevil, which had been effectively controled during the period of high rates and frequent applications of organophosphate pesticides in the late '60s and early '70s, reappeared. In addition, western flower trips, whiteflies, aphids, and spider mites, previously unknown pests, appeared as major cotton insect problems.

History Repeats

By mid-August 1985, tobacco budworm populations in the Brazos Valley had reappeared, and maximum labeled dosages of pyrethroids at five- to seven-day intervals were required tor their control. Data collected by Dr. Bill Plapp (professor of entomology at Texas A&M University and a leading resistance researcher) showed that the tobacco budworm had developed resistance to the synthetic pyrethroids in 1985 and that this resistance was continuing to increase. I had some budworm control problems in late 1986, but the crop was near enough to maturity to terminate with Prep and defoliants. Late season pyrethroid tests at high rates convinced me that we did have resistant tobacco budworm populations in the Brazos Valley of Texas. By July 1987, the synthetic pyrethroid insecticides had become totally ineffective for controlling tobacco budworms in Central Texas. As a result, insect control costs soared because high rates of OP-insecticides were required to finish the crop year. Insect damage was high, resulting in lowered cotton yields and grades. Laboratory assays confirmed high resistance levels to all synthetic pyrethroids.

Resistance Management Strategy

Resistance to the pyrethroid insecticides by tobacco budworms made future cotton production and insect control uncertain. To counter this crisis, we formulated an Insecticide Resistance Management Program for 1988. The program involved organizing area farmers, consultants, chemical dealers, and applicators to agree with and comply to an Insect Resistance Management Strategy (IRMS).

The Insect Resistance Management Strategy integrated the following practices:

1. Changing cotton production practices from a long season growing period to short season early maturity by:

   a. Using short season, fast fruiting, early maturing varieties.
   b. Reducing nitrogen fertilizers by one-half.
   c. Completely controlling early season insect pests to maximize early fruit set.
   d. Irrigating two weeks earlier than usual.
   e. Using harvest aid chemicals--Prep and Dropp--to terminate the mature crops as soon as possible.

2. Prohibiting the use of synthetic pyrethroids during the first 90 days of growing season (emergence to first bloom) and the last 30 days before maturity. Alternate chemistries were recommended during the pyrethroid free periods, i.e., OP, carbamate and biological insecticides during mid-season.

3. Monitoring population with insect traps.

4. Frequent, thorough field scouting to determine infestation levels and economic thresholds.

5. Accurate and precise timing of insecticide applications.

Result of Resistance Management Program

During 1988 all farmers in the Brazos Valley complied to the recommended IRMS, especially delaying the use of synthetic pyrethroids until the early bloom stage. As a result, pyrethroid resistance level in tobacco budworm populations declined significantly when compared to the 1987 levels. Not only was the tobacco budworm more easily controlled, the cost of insect control in 1988 was significantly lowered while maintaining normal cotton yields. As a result, we continued to follow the IRMS in 1989 and 1990 with continued success. Tobacco budworms were economically controlled without yield reductions. However, the situation changed in 1991. Several farmers and/or consultants became complacent and applied synthetic pyrethroids during the early season period before the first bloom stage. Results were devastating. Due to resistance, tobacco budworm populations in mid-July could not be controlled with the pyrethroids. Insect control for the remainder of the season became very expensive, and some farms had yield reductions.

Although the IRMS was successful for reducing tobacco budworm resistance during 1988, 1989 and 1990, secondary pests such as trips, aphids, spider mites and whiteflies, continued to be a problem following pyrethroid applications. Control treatments for these pests greatly increased costs and reduced profits.

Conclusions

Our experiences in the Brazos Valley have shown that Insect Resistance Management Strategies (IRMS) can be highly effective and practical for cotton and other crop production. However, to be successful, an IRMS must include total cooperation and compliance by all farmers in a geographic area large enough to affect the total population of insect pests and their natural enemies. The current problems related to use of synthetic pyrethoids including insect resistance and secondary pest outbreaks must cause us to consider a complete ban of their use or restricting their use to 1-2 applications per season per crop. Future Insect Resistance Management Strategies should focus on Diversified Integrated Crop Management (DICM) to minimize crop damage due to insects. The main objectives of DICM should be the replacement of chemical pesticides with cultural practices and biological materials that maximize control as harmoniously as possible with nature.

Action Area I: Host Plant Resistance Presentation

RECENT DEVELOPMENTS IN *BACILLUS THURINGIENSIS* RESEARCH AND USE OF TRANSGENIC PLANTS FOR CONTROL OF *HELIOTHIS VIRESCENS* AND *HELICOVERPA ZEA*

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Transgenic crops expressing endotoxin proteins of *Bacillus thuringiensis* represent exciting new opportunities to enhance biological control and encourage improved systems of integrated pest management for *Heliothis virescens* and *Helicoverpa zea*. Although several crop plants have been genetically engineered to express endotoxin proteins (Gasser and Fraley 1989), most current research relative to *H. virescens* and *H. zea* emphasizes transgenic cottons expressing endotoxin proteins (BT cotton). Commercialization of BT cotton is expected in the mid- to late-1990s (Deaton 1991). Timely development of management systems that are designed to efficiently utilize this new technology and preserve the insecticidal activity of endotoxin proteins is critical to cotton production and the capabilities of future insect management programs in the United States. Current systems of insect control largely based on the use of chemical insecticides are failing. Pyrethroid resistance is widely distributed in *H. virescens* populations across the United States (Plapp et al. 1990, Elzen et al. 1992), and growers are anxious for cost effective alternatives. In some geographic regions, like the Midsouth, *H. virescens* populations exhibit measurable levels of resistance to all available classes of insecticide chemistry (Elzen et al. 1992, 1994). In 1992, some growers spent more than $100/acre on the control of cotton insects (Head 1993). Preliminary estimates for the 1993 cotton crop indicate that growers may be spending higher amounts than those reported during 1992.

Although integrated pest management (IPM) programs have been emphasized for cotton insect control for more than 30 years (Bottrell and Adkisson 1977), current systems still rely heavily on the use of chemical insecticides (Luttrell 1994). Numerous alternatives to chemical control of *H. virescens* and *H. zea* on cotton have been researched and developed to varying levels, but few have been implemented in production agriculture. Commonly researched alternatives include numerous biological control approaches, development of insect resistant germplasms through traditional crop breeding methods, release of sterile insects as autocidal control agents, management of early season host plants to suppress population growth of the phytophagous species utilizing the plants, and use of microbial insecticides. The status of these alternatives were reviewed in-depth by numerous authors of a new Cotton Foundation Reference Series book to be published in 1994 (King and Phillips 1994). Much of the previous research concentrated on the use of microbial insecticides for control of *H. virescens* and *H. zea*, especially efforts to utilize *B. thuringiensis* and the nuclear polyhedrosis virus of *Heliothis*. Unfortunately, most of the research produced erratic results. While the conceptual advantages of regulating pest populations with these ecologically-sound, alternative-control measures were established, actual implementation of alternative control measures for the management of *H. virescens* and *H. zea* in cotton has been limited.

Reducing insecticide use on cotton is a difficult task because of the complex interrelationships of a diversity of phytophagous and entomophagous arthropods associated with the crop. Insecticide applications directed at several early-season pests, particularly *Anthonomus grandis grandis*, *Lygus lineolaris* and *Pseudomoscelis seriatus*, often disrupt populations of natural enemies which provide effective regulation of *H. virescens* and *H. zea*. Continued, subsequent applications directed at *H. virescens* and *H. zea* essentially eliminate the natural enemies in some cotton production systems. Other crops with less intense use of chemical insecticides may actually be more suitable environments to implement alternative control measures, but the magnitude of the pest problems and the intensive use of insecticides on cotton make the crop an attractive target for public-supported research and private industry investments in potential commercial products. Based on insect loss data reported annually at the Beltwide Cotton Conferences (Head 1991, 1992, 1993), cotton growers in the United States apply more than 51 million acre applications (1 acre application = 1 acre treated 1 time) of insecticide each

year and spend more than $420 million on cotton insect control. A major portion of these applications (39%) and a major fraction of total control costs (42%) are directed at *H. virescens* and *H. zea*. In some regions of the United States, *H. virescens* and *H. zea* may be the target of more than 50% of the total number of insecticide applications made to cotton. Because of the size of this potential commercial market for effective pest control and because of the historical information available on *B. thuringiensis*, BT cotton will be one of the first products of genetic engineering released to consumers.

This report examines current research on BT cotton and provides some speculation as to its role in future management systems for *H. virescens* and *H. zea*. An emphasis is placed on defining research needs. Because much of the technical background for BT cotton is associated with historical information on foliar application of *B. thuringiensis*, an overview of important issues related to the control of *H. virescens* and *H. zea* with foliar applications of *B. thuringiensis* is included.

Bacillus Thuringiensis

Historical Information. The insecticidal activity of *B. thuringiensis* has been known for ca. 100 years (see reviews in Heimpel 1967, Burgess and Hussey 1971, and Burgess 1981). Commercial preparations of the soil-borne bacterium were developed for insect control as early as the 1950s. Basic research during the 1960s and 1970s provided information on the various insecticidal toxins produced by the bacteria and categorized the different modes of actions associated with the exo- and endotoxins (Burgess and Hussey 1971 and Burgess 1981). Howard Dulmage with the USDA-ARS provided leadership in classifying the various strains of *B. thuringiensis* on the basis of H-serotypes and types of insecticidal crystals produced by the various strains (Dulmage 1981). Dulmage was also instrumental in establishing a standardized potency for commercial products based on international units (Dulmage 1981).

Prior to the introduction of the pyrethroid insecticides for control of *H. virescens* and *H. zea* in the late 1970s, a great deal of research was devoted to the development of microbial insecticides for use on cotton. Commercial products were widely tested with varying results. Once the highly efficacious pyrethroids were available to growers, interest in microbials declined and most of the applied research ceased. However, several important relationships between *B. thuringiensis* and the heliothines were established during this period of active research. Several researchers (Ignoffo et al. 1977, Dulmage 1981, Luttrell et al. 1982 and others) documented that different species of Lepidoptera vary in their susceptibility to different preparations of *B. thuringiensis*. Ignoffo et al. (1977) compared the activity of a *kurstaki* strain of *B. thuringiensis* against 6 species of Lepidoptera. They found that *Anticarsia gemmatalis* and *Plathypena scabra* were 10 fold more susceptible than *Trichoplusia ni* which was approximately equal in susceptibility to *Pseudoplusia includens*. *Spodoptera exigua* and *H. zea* were 3 fold less susceptible than *T. ni*. Luttrell et al. (1982) reported that mortality of neonate *H. virescens* and *H. zea* larvae were more susceptible to spray deposits of *B. thuringiensis* on cotton than were second instars. They also reported that *H. zea* was significantly more tolerant of the *B. thuringiensis* deposits than were *H. virescens*. Table 1 summarizes results of several field studies conducted by W. C. Yearian and co-workers at the University of Arkansas from 1974 to 1976 prior to the introduction of the pyrethroid insecticides (Yearian et al. 1980). These studies were conducted during periods of high population densities of *H. virescens*. Yield of plots treated with standard chemical insecticides were 3.5 fold higher than those of the untreated checks. This represented significant insect pressure given that the standard insecticides were organophosphorous insecticides which were declining in efficacy because of insecticide resistance. If the standard insecticides had been the pyrethroids which were first commercialized in the late 1970s, the yield ratios would have been much higher for the standard treatments. In these studies, applications of *B. thuringiensis* provided measurable levels of control, but the control was less than that provided by the standard chemical insecticides. Application of chlordimeform in combination with *B. thuringiensis* increased efficacy of the microbial. In subsequent experiments at Mississippi State University over a 3 year period, R. G. Luttrell and co-workers found that application interval was a significant factor influencing efficacy of microbial insecticides (Table 2). Applications of *B. thuringiensis* applied on a 5-day schedule resulted in cotton yields no higher than those of the untreated check (yield ratio of

treatment/check of only 1.01). When applications were made every day over a 20 to 30 day period, a 65% increase in yield was observed. The effect of application interval was even more obvious with *Heliothis* NPV where the efficacy of virus treatments applied on 1-day intervals were comparable to the efficacy of the pyrethroid insecticide standard applied on a 5-day schedule.

Some of the previous research provided insight into issues relevant to the efficacy of BT cotton and new commercial products of *B. thuringiensis*. Research by Smith and Hostetter (1982) suggested that the type of plant receiving spray deposits of *B. thuringiensis* could alter the amount of control obtained (i.e. there was an interaction between the bacterium and the plant tissue). Mortality of *Trichoplusia ni* and *H. zea* larvae fed *B.*

Table 1. Efficacy of *B. thuringiensis* for control of *H. virescens* and *H. zea* in cotton small plots in Arkansas from 1974 - 1976 (summarized from Yearian et al. 1980).

	Efficacy Ratio[a]		
Treatment[b]	Larval Density	Fruit Damage	Yield
B. thuringiensis	0.75	0.69	1.64
B. thuringiensis + chlordimeform	0.56	0.41	3.01
chlordimeform	0.56	0.50	2.47
standard larvicide	0.37	0.37	3.53

[a] Efficacy ratio equals observation in treatment divided by observation in untreated check.
[b] Teatments applied at recommended rates. *B. thuringiensis* rates from 0.25 to 0.5 lb/acre (16,000 I.U./lb).

Table 2. Effect of spray interval on efficacy of *B. thuringiensis* and *Heliothis* nuclear polyhedrosis virus (NPV) for control of *H. virescens* and *H. zea* in cotton small plots.

		Efficacy Ratio[a]	
Treatment[b]	Spray Interval	Damaged Fruit	Yield
B. thuringiensis	1 day	0.51	2.04
B. thuringiensis	3 day	1.00	1.42
B. thuringiensis	5 day	1.15	1.31
Heliothis NPV	1 day	0.81	1.65
Heliothis NPV	3 day	0.98	1.61
Heliothis NPV	5 day	1.25	1.01
Pyrethroid	5 day	0.41	2.08

[a] Efficacy ratio equals observation in treatment divided by observation in untreated check.
[b] *B. thuringiensis* was applied at a rate of 1.0 lb/acre of a 16,000 I.U. (international unit)/mg commercial product. *Heliothis* NPV was applied at 40 L.E. (larval equivalents)/acre. The pyrethroid standard was either fenvalerate or permethrin and was applied at a rate 0.1 lb a.i.(active ingredient)/acre.

thuringiensis on soybean or cabbage foliage died at higher rates than those fed on cotton. This may indicate the interaction of secondary plant compounds with insecticidal action of the bacteria. It is possible that different cultivars within the same crop species could interact differently with spray deposits of *B. thuringiensis* or plant produced insecticidal proteins. This area is currently being investigated by G. W. Felton and co-workers at the University of Arkansas. Interested readers should refer to Felton and Dahlman (1984) and Ludlum et al. (1990).

Ignoffo and Falcon (1978) and Smith (1981) reviewed research conducted to improve microbial preparations through the addition of various spray adjuvants and unique application procedures. Although many of these efforts, particularly the use of spray adjuvants and feeding stimulants, tended to increase insect mortality, resulting larval mortality was still less than that obtained with chemical insecticides. Perhaps the data reported iby Phillips et al. (1979) (Table 3) and Yearian and Phillips (1983) (Table 4) best summarizes the level of control of *H. virescens* and *H. zea* expected in field situations. The data of Phillips et al. (1979) showed a rate response to a commercial *B. thuringiensis* product, Dipel®, and the differential susceptibilities of *H. virescens* and *H. zea* to the bacterial insecticide. At economical use rates less than 1.0 lb/acre (e.g. typical use rates were 0.25 and 0.5 lb/acre), expected moralities of 30% or less would have been expected. Neonate larvae were more susceptible than 2nd instars. Interestingly, foliar applications of new *B. thuringiensis* products are currently economical at use rates as high as 8 B.I.U.(billion (10^9) international units)/acre. Equivalent rates of the Dipel used in 1979 (16,000 I.U./mg) would be 1.1 lb/acre. The 1979 data would suggest that 8 B.I.U.'s/acre would provide larval mortalites between 10 and 40% depending upon the age of larvae and species targeted. The data of Yearian and Phillips (1983) corroborate the 1979 report and confirm the lack of adequate crop protection when the microbials were used season long for the suppression of *H. virescens* and *H. zea*.

An overall analysis of the research conducted during the 1970s indicates that *B. thuringiensis* had activity against the heliothine pests of cotton, but the level of insecticidal activity provided was low. Major advantages to the use of both *B. thuringiensis* and *Heliothis* NPV were their abilities to selectively reduce pest populations and preserve the natural enemies that were also important in regulating pest population growth. It is quite possible that the regulating influence of natural enemies plus the selective mortality provided by the microbials could be more effective in reducing population growth of target pests than the high killing power of chemical insecticides in the absence of natural mortality. Although these concepts were and are still ecologically valid, the diversity of insect pests in cotton and the traditional need for chemical insecticides has largely negated the development of management systems capable of capitalizing on the benefits of natural control (Luttrell 1994).

Table 3. Mortality of neonate and 2nd instar larvae of *H. virescens* and *H. zea* exposed to cotton treated with varying rates of a commercial *B. thuringiensis* product, Dipel® (abstracted from Phillips et al. 1979).

	Mean % Corrected Mortality			
	H. virescens		*H. zea*	
Treatment[a]	Neonate	2nd Instar	Neonate	2nd Instar
Dipel 1 lb/acre	34	19	22	9
Dipel 2 lb/acre	48	23	33	8
Dipel 8 lb/acre	59	43	53	15
Dipel 20 lb/acre	79	69	62	61

[a] Dipel contained 16,000 I.U. (international units)/mg.

Table 4. Comparison of yields from cotton treated with *B. thuringiensis*, *Heliothis* NPV, and a standard organophosphorous (OP) insecticide (abstracted from Yearian and Phillips (1983))[a].

Year	*B. thuringiensis*	*Heliothis* NPV	Standard Insecticide
1967	=	=	=
1969	=	=	=
1971	=	=	=
1972	(no data)	=	>
1973	≥	>	>
1974	=	=	>
1975	=	=	>
1976	=	=	>
1977	=	≥	>

[a]= refers to yields statistically equal to untreated check
> refers to yields statistically greater than untreated check
≥ refers to yield numerically but not statistically greater than untreated check

The lack of curative, efficacious control of *H. virescens* and *H. zea* with foliar applications of *B. thuringiensis* is largely associated with the low persistence of the microbials on cotton foliage and the need for larvae to consume treated plant tissue (Phillips et al. 1979, Luttrell et al. 1982, Ali et al. 1993). Atomized spray deposits are generally too variable to insure a high probability of larvae contacting toxic dosages of insecticide (Luttrell and Smith 1990), especially with fruit feeding insect like *H. virescens* and *H. zea*. Problems with low persistence and variable contact with spray deposits were eliminated with development of transgenic plants that constitutively express the insecticidal toxin in all plant structures throughout the growing season (Meeusen and Warren 1989).

Recent Research with *Bacillus thuringiensis*. During the 1980s and 1990s basic research with *B. thuringiensis* accelerated, particularly in areas associated with molecular genetics. Application of *B. thuringiensis* to production agriculture and its inherent genetic variability stimulated interest in the organism for genetic engineering projects. In the 1970s and 1980s, a great deal of information was generated on the activity of the endotoxins produced by *B. thuringiensis*, and genetic techniques were developed to better describe the genetic variation within the species. From these capabilities, an improved classification system was developed that described the different insecticidal proteins on the basis of genetic material (DNA) and host range of the insecticidal proteins (Hofte and Whiteley 1989). An overview of the classification system is given in Table 5. CryI and CryII genes encode proteins that have insecticidal activity against Lepidoptera. CryIII genes encode proteins active against Coleoptera, and CryIV genes encode proteins active against Diptera. During the 1980s, strains with activity against Coleoptera, primarily against the Colorado potato beetle, and strains with activity against Diptera, particularly mosquitoes, were developed and commercialized (Gawron-Burke and Baum 1991). Basic research on the genetic diversity within *B. thuringiensis* continues and is the "backbone" of many biotech companies. Because of the ability of some bacteria to transfer genetic material from one strain to another via transcon-

jugation and recombinant techniques, the possibilities for increasing genetic diversity in *B. thuringiensis* are almost infinite. Some industry collections of *B. thuringiensis* isolates may number more than 1,000, and scientists are constantly searching for new strains and creating new combinations of the genome through transconjugation and recombinant DNA techniques. These different insecticidal proteins serve as the source of new insecticidal proteins for future transgenic plants.

Table 5. Host range of different *B. thuringiensis* insecticidal protein genes (abstracted from Gawron-Burke and Baum (1991)).

Gene Type	Host Range
cryIA(a), cryIA(b), cryIA(c), cryIB, cryIC, cryID, cryIE, cryIF	Lepidoptera
cryIIA, cryIIB	Lepidoptera/Diptera
cryIIIA, cryIIIB, cryIIIC	Coleoptera
cryIVA, cryIVB, cryIVC cryIVD	Diptera
cytA	Diptera

Perhaps the most exciting development in agriculture in the last few decades is the ability of scientists to genetically transfer genetic material from one organism to another. Gasser and Fraley (1989) reviewed the potential applications of genetically engineered plants to agriculture. Information and capabilities in plant transformation are expanding dramatically because of commercial interest in the technology. Reviews are often outdated before they are printed.

In addition to advances in molecular genetics and genetic engineering, some renewed interest in the efficacy of foliar applications of *B. thuringiensis* for control of *H. virescens* and *H. zea* on cotton developed in the late 1980s and 1990s because of the declining efficacy of chemical insecticide. The number of commercial products has greatly increased and the volume of material applied to cotton in the past few years is probably more than the amount applied during the past 15 years. Research is being conducted to verify the activity of these new strains and relate expected results to those obtained in the 1970s. Research in our laboratory has emphasized a life table approach to understanding the role of *B. thuringiensis* in managing *H. virescens* and *H. zea*. Although the activity of some commercial products is higher than that available in the 1970s, the limiting factors appear to be the same. Low persistence and the fact that target insects must consume the material still limit efficacy against fruit feeding insects on cotton. During the past few years, we confirmed or found the following: (1) activity of new *B. thuringiensis* products vary in activity against different pest species (*H. virescens*, *T. ni*, and *Pectinophora gossypiella* are relatively susceptible; *H. zea* and *P. includens* are slightly more tolerant; *Spodoptera frugiperda* and *Spodoptera exigua* are very tolerant), (2) mortality of *H. virescens* larvae exposed to deposits of *B. thuringiensis* on cotton is influenced by larval age, length of incubation between contact and desired effect, and rate of material applied, (3) activity of different commercial products vary differentially against the different pest species targeted (i.e., one product may be preferred to control *H. virescens* but less preferred for *H. zea*), (4) there is a need to standardize activity of commercial products for extension recommendations, and (5) levels of mortality observed in the field still range from 20 to 60% depending on

various factors. Overall, recent results still illustrate the low insecticidal activity of the microbials and confirm the need to couple their use with other control measures like conservation of natural enemies.

Resistance to *Bacillus thuringiensis*. During the late 1980s, a new area of interest developed relative to *B. thuringiensis*. Resistance in laboratory selected colonies of *Plodia interpunctella* and *H. virescens* were reported (McGaughey and Whalon 1992). Before 1985, the thinking was that resistance would not likely develop to the endotoxins produced by the bacterium. There are now documented reports of resistance to *B. thuringiensis* in at least 8 species: *Plutella xylostella*, *P. interpunctella*, *Cadra cautella*, *H. virescens*, *Leptinotarsa decemlineata*, *Aedes aegypti*, *Culex quinquefasciatus*, and *Homoeosoma electellum* (McGaughey and Whalon 1992). Resistance in field populations of *P. xylostella* were confirmed in Hawaii (Tabashnik et al. 1990). Except for *L. decemlineata* and *P. interpunctella*, reported cases of resistance are associated with laboratory selection experiments. The expected commercial release of transgenic plants with continuous expression in all plant tissues intensifies concerns for resistance development because of the expected higher selection pressures provided by the insecticidal plants. Development of resistance management strategies is being pursued, and concerns for resistance to transgenic plants is one of the most intensely debated issues in agriculture. In our laboratory, we are screening different populations of *H. virescens*, *H. zea*, *P. includens* and *S. exigua* to establish base-line data for future monitoring efforts. Variations in the response of different populations to purified HD-1 (truncated cryIA(b)) and HD-73 (cryIA(c)) endotoxin proteins have been observed (Table 6), but

Table 6. Range in LC-50's for different populations of *H. virescens*, *H. zea*, *P. includens*, and *S. exigua* exposed to purified HD-1 (cryIA(b)) and HD-73 (cryIA(c)) endotoxin proteins in diet incorporation assays.

	Number of Colonies Tested	Range in LC-50's Expressed as ug of protein/ml of diet
	HD-1 (cryIA(b)	
H. virescens	14	0.01 - 0.48
H. zea	14	0.22 - 424.90
P. includens	1	0.66
S. exigua	5	41.00 - (55% survival at 30 ug/ml dose)
	HD-73 (cryIA(c)	
H. virescens	18	0.01 - 0.14
H. zea	20	0.02 - 36.57
P. includens	2	0.21 - 0.48
S. exigua	5	3.38 - (85% survival at 30 ug/ml dose)

[a]Colonies of *H. zea* with LC-50's higher than 23.68 with HD-1 and 6.6 with HD-73 represented laboratory selected colonies. All other colonies were not selected.
[b]With *S. exigua* significant survival was observed at a doses of 30 ug/ml with some colonies. Because of the cost of the protein, higher doses were not used and regression equations were not developed.

the biological significance of this variation is currently unknown. Our results largely corroborate those of Stone and Sims (1992) who reported variation in the response of *H. virescens* and *H. zea* to HD-73 and HD-1 proteins across populations collected in different geographic regions of the United States.Research is currently being conducted to evaluate different resistance management options and to better understand the biological significance of the variation across populations in response to the endotoxin proteins. Behavioral research is being conducted at several laboratories to understand the avoidance behavior of insects for *B. thuringiensis* (in both foliar products and transgenic plants) and the effects of different behaviors on the ultimate utility of various management strategies designed to create a refugia for susceptible genotypes within the population.

Transgenic Cotton Expressing Endotoxin Proteins of *Bacillus Thuringiensis*

Crop plants that have been genetically altered to express insecticidal proteins of *B. thuringiensis* offer a unique, new method of delivering insecticide to target pests. Meeusen and Warren (1989) provided an early review of the potential effects of genetically engineered crops on insect control. Advantages reported were: (1) growers would be less dependent on favorable weather conditions for application of insecticides because insecticidal activity would be continuously expressed and not altered by inclement weather, (2) lower locations of the plant canopy where insecticide sprays cannot be dependably deposited would be protected from insect damage, (3) the need to scout crops would be reduced because of the continuous expression of insecticidal activity, (4) the costs of spraying crops would be reduced, (5) the cost of developing commercial insect-resistant crops would be less than that required to develop a new chemicalinsecticide, (6) spray drift and ground water contamination would be reduced, (7) adverse side effects on non-target organisms would be reduced, and (8) monitoring of crops for insecticidal residues would be simplified. Potential limitations envisioned were the likely selection for resistant pest populations and regulatory hurdles associated with safety and patent protection of a new technology. Meeusen and Warren (1989) was written from the perspective of commercial industry executives. Most of the advantages predicted were associated with projected reductions in the use of chemical insecticides. Although these potential advantages and limitations are highly speculative and the development of transgenic crops expressing *B. thuringiensis* proteins is in its infancy, considerable information has been generated in the past few years, particularly with BT cotton, confirming these initial projections. Within the next few years, it is likely that research on transgenic crops expressing insecticidal proteins will dramatically expand and additional advantages and limitations of the technology will be evident. Advantages of the technology seen from the perspective of a research entomologist interested in population management of *H. virescens* and *H. zea* include: (1) expanded opportunities for use of biological control, particularly conservation of natural occurring predators and parasites, (2) improved control of some lepidopteran pest species not effectively controlled by chemical insecticides, (3) demonstration of a revolutionary, environmentally-safe application method for delivering a toxicant to a target pest population, and (5) some specific, unique opportunities to implement large-scale, area-wide programs of pest population suppression.

The initial, experimental lines of BT cotton failed to express insecticidal activity at sufficient levels to provide economic control of *H. virescens* (Benedict et al. 1992, Jenkins et al. 1990). Improved transformations of the genetic cotton resulted in experimental lines that expressed higher levels of the insecticidal protein (Perlak et al. 1991) and provided excellent control of *H. virescens* and good control of *H. zea* (Benedict et al. 1993, Jenkins et al. 1993). Mortality rates of *H. virescens* exposed to BT cotton are generally as high (i.e. greater than 85% mortality) or higher than those expected from efficacious insecticides targeted at susceptible pest populations (Luttrell et al. 1987). DeSpain et al. (1993) found no larvae surviving to pupation and no adult emergence when *H. virescens* larvae were placed on BT cotton as 1st, 2nd, 3rd or 4th instars and confined for the remainder of the larval development period (Table 7). Pupation rates for similar aged larvae exposed to non-BT cotton ranged from 15 to 51%. Adult emergence rates ranged from 10 to 25%. When 5th instar larvae were placed on the BT cotton, 51% survived to pupation and 29% successfully emerged as adults. For 5th instars placed on non-BT cotton, the pupation rate was 81% and the adult emergence rate was 49%. Jenkins et al. (1993) exposed larvae to cotton cotyledons, seedling stems, first true leaves, terminal leaves, old leaves, squares without bracts, squares without bracts and petals, and petals of non-BT and BT cotton. They found survival rates of larvae

ranging from 49 to 69% on the non-BT cotton. On BT cotton, larvae survival ranged from 0 to 8% (Table 7). In a similar study, Jenkins et al. (1993) reported survival rates of larvae after 6 days of exposure to terminals of different BT cotton lines from 4 to 16% (Table 7). Survival on non-BT cotton was 81%. Benedict et al. (1993) studied survival and behavior of *H. virescens* and *H. zea* on several BT cotton lines. Survival rates of *H. virescens* ranged from 0 to 5% depending on the experimental line evaluated (Table 7). Survival of *H. zea* was

Table 7. Survival of *H. virescens* larvae exposed in BT cotton in several published experiments.

Age or Size of Larvae	Length of Exposure	Percent Survival	Reference
1st instar	until pupation	0	DeSpain et al. 1993
2nd instar	until pupation	0	DeSpain et al. 1993
3rd instar	until pupation	0	DeSpain et al. 1993
4th instar	until pupation	0	DeSpain et al. 1993
5th instar	until pupation	51	DeSpain et al. 1993
1st instar	6 days	4-16	Jenkins et al. 1993
1st instar	until pupation	0-5	Benedict et al. 1993
1st instar	2 days	34-48	Mascarenhas et al. 1994
1st instar	2 days/pupation[a]	10-21	Mascarenhas et al. 1994
1st instar	1 day	43	Mascarenhas et al. 1994
1st instar	2 days	16	Mascarenhas et al. 1994
1st instar	3 days	14	Mascarenhas et al. 1994
1st instar	3 days	11	Mascarenhas et al. 1994
1 day old	2 days	38	Mascarenhas et al. 1994
2 day old	2 days	49	Mascarenhas et al. 1994
4 day old	2 days	72	Mascarenhas et al. 1994
1 day old	2 days/pupation	15	Mascarenhas et al. 1994
2 day old	2 days/pupation	28	Mascarenhas et al. 1994
4 day old	2 days/pupation	46	Mascarenhas et al. 1994

[a] After 2 days of exposure, larvae were transferred to artificial diet and observed for survival at pupation.

slightly higher, ranging from 1 to 19%. Survival of *H. virescens* on non-BT cotton was 60% and of *H. zea* on non-BT cotton was 31%. Using slightly different methodology, Mascarenhas et al. (1994) individually caged larvae on plant structures of non-BT and BT cotton in large field plots. First instar (neonate) larvae caged on different cotton plant structures for 2 days survived at rates between 34 and 48% (Table 7). Survival rates on non-BT cotton were 37 to 81%. Following the 2 day exposure period, larvae were transferred to artificial diet and observed at pupation. Resulting mortality levels ranged from 10 to 21%. Survival rates as high as 52% were observed for insects that had been initially exposed to non-BT cotton. When the length of exposure to BT cotton varied from 1 to 4 days, survival rates decreased from 42 to 11% (Table 7). When older larvae (2 and 4 day old) were exposed to the plant structures, survival increased from 38% for neonates to 72% for 4 day old larvae (Table 7). Subsequent observations of the same larvae transferred to diet showed pupation rates between 15 and 46% (Table 7). Collectively, the results of these studies demonstrate the high insecticidal activity of BT cotton against *H. virescens* and *H. zea*. Small plot field experiments corroborate these findings and illustrate the ability of the insecticidal plants to effectively protect crop yield (Jenkins et al. 1993). Insecticidal activity of BT cotton against *H. virescens* and *H. zea* is high.At this time, research with BT cotton has confirmed high efficacy against *H. virescens* and *H. zea*. The types and scope of research projects associated with BT cotton are rapidly expanding with the increased availability of seed and increased experience in working with a highly regulated product of genetic engineering. In 1994, many of the extension entomologists in cotton growing regions of the United States will have demonstration plots. Numerous critical questions remain to be answered, particularly those associated with production level environments. Research is underway to understand the potential impact of BT cotton on the total arthropod complex within a cotton production environment. The scale of the research is important because of the surrounding influence of insecticide sprays. Some data have been obtained in 5 acre plots in the delta production region of Mississippi. BT cotton does not appear to have any direct effect on non-target organisms, although some density related effects seem to be present. If BT cotton reduces the number of lepidopteran insects within a system, it is logical that the predatory and parasitic species that utilize lepidopterans as a host would be reduced. This appears to be the case, but more data are needed.

Research projects have also been initiated to better understand various aspects of *H. virescens* biology that are critical to resistance management and the long-term utility of this highly efficacious insect control tool. Understanding the avoidance behavior of larvae exposed to *B. thuringiensis* and/or BT cotton is the goal of current research at Mississippi State University, the University of Arkansas, North Carolina State University, and Texas A&M University. Researchers are particularly interested in larval behavior as it relates to the seed mixture strategy for creating a refugia for susceptibility within the system. On-going research is further describing the variation in susceptibility of different populations of lepidopteran pests to commercial formulations and purified proteins of *B. thuringiensis*. Additional studies are attempting to relate this variation to potential survival on BT cotton. Researchers are also searching for resistant genes and attempting to estimate the expected frequency of these resistant genes in indigenous populations of *H. virescens*. Considerable basic research is underway around the world to better understand the mode of action of different endotoxin proteins in both susceptible and resistant insects. Industry groups are actively searching for alternative insecticidal proteins for potential replacement of current genes being used in transgenic plants.

Although transgenic technology is an exciting new area of biology and tremendous amounts of new research are producing information, some of the factors limiting effective insect control need more attention. Much of the success or failure of the transgenic plants expressing insecticidal proteins will be determined by the mechanisms by which the technology is implemented. To effectively transfer this valuable technology to production agriculture, entomologists need more information on the biology and ecology of the target pest species. The potential advantages of the insecticidal plants (i.e., reduced insecticide, expanded opportunities for biological control, unique opportunities for population regulation, etc.) warrant additional investments in research to better understand the pest-crop systems targetedfor commercialization of the transgenic products. Theoretical models have projected potential problems with resistance development and have defined research areas that are critical to the long-term success of this technology, but there is an urgent need for more empirical research to confirm or refute critical assumptions in the models.

The development of pest populations resistant to endotoxin proteins is critical to the long term success of the plants engineered to express these insecticidal proteins. However, it is not the only limiting variable governing success of the technology. Information is also needed on the relationships of the technology to the need and ecological impact of other control measures directed at other pests. For example, applications of insecticide for *Lygus* spp. may negate any advantages associated with reduced inputs of insecticide for control of *H. virescens* or *H. zea*. Applications of insecticide directed at *H. virescens* and/or *H. zea* may provide indirect suppression of other phytophagous species. If these treatments are removed, additional insecticide treatments may be needed to control these previously unrecognized pests. On the other hand, some of the ecologically disruptive effects of applications directed at *H. virescens* or *H. zea* on natural enemies that control other phytophagous pests may be avoided. Answers to these issues will remain as speculation until they are addressed in large plot experiments.

In a broader ecological sense, more information is needed on the movement and ecology of polyphagous species attacking several agricultural crops within the same agroecosystem. When one considers that BT cotton and BT corn (corn genetically modified to express endotoxin proteins) may be planted within the same agricultural area, it is easy to understand the importance and potential impact of management decisions made in one crop on the efficacy of management actions in another crop. With *H. zea*, this particular example may be a real concern, because it is commonly found at high densities on both crops. Other pest-crop examples exist. Soybean loopers (*P. includens*) in Mississippi are effectively controlled with foliar application of *B. thuringiensis*. Populations of soybean looper are higher in areas where soybean is grown adjacent to cotton. The soybean looper moths utilize cotton as a source of nectar and which presumably results in an increased fecundity (Burleigh 1972). In recent years, soybean looper larvae have become more commonly found in cotton suggesting that some oviposition may occur within cotton fields. If these cotton fields are planted to BT cotton and provide sufficient selection for resistant genotypes, the efficacy of the foliar applications in soybean could be dramatically reduced. These potential interactions of polyphagous pests and sister crops within a production area illustrate the need for more research on community ecology.

Immediate research needs that could impact the design of resistance management strategies and alter implementation procedures include a need for information about larval and adult movement and a need for information on potential gene flow (as well as gene frequencies) which would allow better estimates of the size of refugia needed for resistance management. Some of the longer term needs include understanding how the population suppression capabilities of this technology could work jointly with other area-wide control measures. The high insecticidal activity of BT cotton may dramatically reduce populations densities of *H. virescens* when it is first planted over significant portions of the cotton acreage in some geographical areas. Costs associated with the release of autocidal control agents are often related to the density of the target pest population. Effectiveness is usually highest when releases are targeted against low pest densities. Wide-spread adoption of BT cotton and subsequent reductions in *H. virescens* populations could create a unique opportunity to target area-wide management strategies against low densities of the pest. As resistance to the BT cotton increases in the pest population, the advantages associated with the timing of autocidal control measures against low pest densities will diminish.

Several other research questions associated with BT cotton on individual fields or farms need attention. Can pest managers capitalize on reduced insecticide inputs and effectively utilize natural enemies for suppressing subsequent pest problems? Too what extent can pest managers depend upon these natural enemies? Given the projected changes in insecticide use, what new pest problems will emerge? What current pest problems are likely to decrease? As new insecticidal plants are developed by industry, a need for unbiased evaluation is needed. The efforts of USDA-ARS scientists to study the ecology of *H. virescens* and *H. zea* over large geographic areas should be continued. The stability of USDA-ARS host plant resistance research has provided the cotton industry with a mechanism to properly evaluate public and private generated germplasm. This effort should be continued with an emphasis on products of genetic engineering. Many USDA-ARS programs may represent the only realistic option for generating continuous information over time spans of several years. These long-term commitments to pest ecology and agricultural science are critical to agricultural production in the United States.

Summary

Transgenic plants expressing endotoxin proteins of *B. thuringiensis* offer a unique opportunity to develop improved management systems for *H. virescens* and *H. zea*. This technology seems to offer a realistic alternative to the use of chemical insecticides, and some reduction in insecticide use with implementation of insecticidal plants seems likely. However, the extent of this reduction is difficult to estimate.

Historical research with *B. thuringiensis* provided a frame-work for development of transgenic plants expressing endotoxin proteins. Within the past decade, amazing advances in molecular biology and genetic engineering have created unique systems of delivering toxicants to target pest populations. Applied research has demonstrated the high insecticidal activity of these genetically altered plants, but more information is needed if we are to effective capitalize on the benefits of this new technology.

Relative to traditional research thrusts of the USDA-ARS, the development of these insecticidal plants may create an ideal environment for the area-wide use of autocidal or biological control technologies. Research on the basic biology and ecology of *H. virescens* and *H. zea* must be continued and emphasized by USDA-ARS scientists. Large-plot or field size studies should be initiated to better understand the impact of BT cotton on reduced insecticide inputs and the relationships to other pest problems within the system. Public scientists should continue to standardize and screen the new insecticidal proteins developed by industry. Investigations emphasizing community ecology, insect movement, and insect mating behaviors should be emphasized.

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VARIABLES IN SPRAY DEPOSITS, EFFICACY, AND DRIFT - A REVIEW

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Application technology involves many variables that affect how the control agent is delivered to a target pest and the amount of material lost or ineffective in controlling the pest. The delivery process involves formulations, transfer to the target, and uptake by the target; the bottom line is how best to effectively and safely deliver a lethal dose. Formulations, application equipment, operational parameters, target parameters, and weather are a few variables that must be considered. More attention needs to be given to where pests are located on plants, the site of action of different pest control agents, where the material should be applied, the dose, and other factors affecting application efficacy and safety. Application requirements are equally important for biological and chemical pest control agents and should be addressed early in the development of new pest control technologies.

Spray Deposition on Plant Foliage

Spray deposition on plant foliage is greatly affected by location in the plant canopy; especially when plants are mature and canopies are closed (Table 1). Droplet size and spray rate both influence the deposition of pest control material on plant leaves. Small droplets tend to deposit more efficiently on small targets such as grass-type plants as shown in Table 2; while large droplets tend to deposit more efficiently on broadleaf plants such as cotton. Kirk et al. (1992a) found that large droplet spectrum sprays gave higher mean deposits on cotton leaves than small droplet spectrum sprays (0.028 and 0.024 μg/cm2, respectively).

Table 1. Spray deposits on cotton for two canopy locations and two spray rates[a]

Spray rate, L/ha	Canopy location	Mean deposit, $\mu g/cm^2$
18.7	Top	0.036
	Midway	0.012
46.8	Top	0.042
	Midway	0.013

[a]After Kirk et al. (1992a).

Table 2. Mean deposit of tridiphane on yellow foxtail for different spray rates and droplet sizes[a].

Spray rate, L/ha	$D_{v0.5}$,[b] μm	Mean deposit, kg/ha
18.7	380	0.13
	264	0.21
46.8	408	0.23
	318	0.35

[a]After Kirk et al. (1989).
[b]Droplet volume median diameter ($D_{v0.5}$).

Small droplets are more readily deflected by air currents and thus tend to be carried around large leaves by air movement. Larger droplets have more momentum and their trajectories are likely to be affected less by air movement; however, air currents produced by a low-flying aircraft tend to move leaves around and flip some leaves over, exposing the leaf bottoms to falling droplets. Spray composed of small droplets deposits more efficiently on the smaller leaves because of the increased number of droplets in a given volume of spray and the fact that small leaves result in smaller air deflections. Large droplets tend to deposit more efficiently on the tops of leaves. Small droplets tend to penetrate deeper into the plant canopy and deposit material on the bottoms of leaves. However, Bouse et al. (1993) found that sprays applied in larger droplets can result in more canopy penetration and deposition on the bottoms of leaves in the top part of the canopy, probably because larger droplets are less affected by evaporation, tend to reach the canopy sooner, and are less likely to drift. Bouse et al. (1993) compared spray deposition on cotton and spray drift from aerial applications for two nozzle types and three airspeeds that produced a wide range of droplet sizes (Table 3). The larger droplets produced by the fan (flat spray) nozzles provided more spray deposit on the tops and bottoms of cotton leaves than did smaller droplets produced by the WhirlJet (hollow cone) nozzles (Table 4).

Table 3. Treatments for spray deposition and drift tests[a].

Nozzle type[b]	Air speed, km/h	Spray press., kPa	Spray rate, L/ha	$Dv_{0.5}$,[c] μm	Spray volume, %	
					$<100\mu$m	$<200\mu$m
Fan	193	207	41.6	418 a[d]	0.18 d	2.62 f
Fan	217	207	37.8	358 b	0.22 d	3.83 e
Fan	242	207	34.1	314 d	0.39 c	6.58 c
WJ	193	290	41.6	333 c	0.42 c	5.44 d
WJ	217	290	37.8	289 e	0.65 b	9.95 b
WJ	242	290	34.1	264 f	1.07 a	17.40 a

[a]After Bouse et al. (1993).
[b]Fan (No. 4020 flat spray) and WJ (1/8B10-8 WhirlJet); Spraying Systems Co.
[c]Droplet volume median diameter ($Dv_{0.5}$).
[d]Means in a column followed by the same letter are not significantly different (P=0.05).

Table 4. Spray deposition on cotton leaves[a].

Nozzle type[b]	Air speed, km/h	Deposit, μL/cm^2				
		Top canopy		Mid canopy		Canopy mean
		Leaf top	Leaf bottom	Leaf top	Leaf bottom	deposit
Fan	193	0.118 ab[c]	0.071 a	0.085 ab	0.021 a	0.074 a
Fan	217	0.124 ab	0.068 ab	0.079 ab	0.005 b	0.069 ab
Fan	242	0.112 ab	0.041 abc	0.099 a	0.010 ab	0.065 ab
WJ	193	0.135 a	0.023 c	0.063 b	0.003 b	0.056 abc
WJ	217	0.093 bc	0.041 abc	0.063 b	0.011 ab	0.052 bc
WJ	242	0.058 c	0.038 bc	0.060 b	0.007 b	0.041 c

[a]After Bouse et al. (1993).
[b]Fan (No. 4020 flat spray) and WJ (1/8B10-8 WhirlJet); Spraying Systems Co.
[c]Means in a column followed by the same letter are not significantly different (P=0.05).

The Aerial Application Research Unit has conducted several studies to evaluate the effects of application techniques on spray penetration into the plant canopy and deposition on both the tops and bottoms of leaves. Results have shown that flying close to the crop, reducing aircraft speed, and using air deflectors attached to the spray boom increase air movement into and within the plant canopy, increasing penetration of spray into the canopy, and tend to deposit more material on the plants.

Constant temperature anemometry measurements were made to quantify the effects of several operational factors on the strength of air currents in the aircraft wake. Results of tests over an aircraft runway indicated that increasing aircraft loading increased mean airflow by 48% and turbulence by 26%, decreasing airspeed from 184 to 144 km/h (115 to 90 mph) increased mean flow by 64% and turbulence by 10%, and decreasing height from 4.5 to 1.5 m above ground increased mean flow 250% and air turbulence by 60% relative to ambient airflows (Franz and Carlton, 1993). Therefore, aircraft height above the crop, aircraft speed, and other factors that affect the downwash of spray-laden air following aircraft passage should be expected to increase canopy penetration and spray deposition on leaves.

An aircraft spray boom equipped with Chimavir winglets (air scoops attached to the boom to deflect air and spray downward) deposited twice as much spray on the bottoms of cotton leaves in the top of the plants as did a conventional spray boom (Kirk et al., 1992b). Similar results (a 52% increase in deposits on the bottom of leaves in the top of the canopy) were obtained by reducing the aircraft speed from 184 to 144 km/h when using a conventional spray boom. However, the increased downward air velocity and air turbulence effects are rapidly dampened out by foliage and there was no difference in deposits on the bottoms of leaves at the mid-canopy level. Mean spray deposits on the tops of leaves ranged from 3 to 8 times those on the bottoms of leaves in the top of the cotton plants and 6 to 10 times those on the bottoms of leaves at the mid-canopy level.

In a similar study (Kirk et al., 1993), spray droplet size and density on water sensitive papers attached to the tops and bottoms of cotton leaves were compared for several types of aerial spray systems including rotary atomizers, winglets, CP nozzles with 90 deg deflectors, and "trumpet" nozzles that direct air and spray downward through tubes attached to the aircraft (Custom Farm Services, Stanfield, AZ). As shown in Table 5, the rotary atomizers deposited higher droplet densities on the tops and bottoms of leaves in the top of the plants as well as on the tops of leaves at the mid-canopy level. However, differences in droplet densities on the bottoms of leaves at the mid-canopy level were negligible. Droplets deposited on the tops of leaves at both the top and mid-canopy plant heights were smaller for the rotary atomizers than for the other treatments.

Treatment Efficacy

While it is well known that droplet size influences drift, the effects of droplet size on efficacy are less obvious. Some pesticides and some formulations are more efficacious when applied in small droplets while others give better pest control when applied in larger droplets (Kirk et al., 1991). In a comparison of Heliothis virescens egg and larval mortality for three different ovacides applied as 210 vs 330 μm Dv.5 sprays, Curacron performed better with the larger droplets while Larvin and Ovasyn provided slightly greater mortality when applied as smaller droplets (Table 6). Spray rate (L/ha) had relatively little effect on larval mortality for Curacron. However, Larvin was more effective applied at 46.8 than at 18.7 L/ha while the reverse was true for Ovasyn. These results were obtained using the same rate of active ingredient (a.i.); therefore, the concentration of a.i. in the spray mixture varied.

Table 5. Mean spray droplet density (#/cm^2) and droplet volume median diameter ($Dv_{0.5}$, μm) at top and mid-canopy locations for six spray treatments[a].

Treatment	Top canopy				Mid canopy			
	Leaf top		Leaf bottom		Leaf top		Leaf bottom	
	#/cm^2	$Dv_{0.5}$	#/cm^2	$Dv_{0.5}$	#/cm^2	$Dv_{0.5}$	#/cm^2	$Dv_{0.5}$
Rotary atomizer 185 km/h, 1.5 m[b]	121	128	1	57	40	94	4	25
Rotary atomizer 145 km/h, 1.5 m	118	138	37	86	32	109	5	32
Winglets 170 km/h, 1.5 m	103	154	38	97	30	122	4	26
CP nozzles 195 km/h, 1.5 m	67	173	13	68	25	139	4	25
Trumpet nozzles 175 km/h, 1.5 m	60	177	19	63	20	144	3	25
Trumpet nozzles 175 km/h, 3.0 m	48	176	10	44	18	141	4	29

[a]After Kirk et al. (1993).
[b]Height of aircraft wheels above ground.

Table 6. Effect of droplet volume median diameter ($Dv_{0.5}$) and ovicide on mean percentage mortality of *Heliothis virescens* eggs and neonate larvae from aerial spray treatments[a].

Ovicide	Eggs		Neonate larvae	
	Droplet size, $Dv_{0.5}$		Droplet size, $Dv_{0.5}$	
	210 μm	330 μm	210 μm	330 μm
Curacron	10.3	12.4	65.2	78.6
Larvin	25.9	19.0	86.0	80.2
Ovasyn	26.4	23.6	62.2	58.9

[a]After Kirk et al. (1991).

In a comparison of nozzle type effects on pyrethroid efficacy, Kirk and House (1992) found that Karate and Asana XL both provided greater mortality of neonate larvae and less damaged cotton fruit when applied as a combination of larger droplets and a higher spray rate, however the efficacy of Asana XL was much more sensitive to these parameters than was that of Karate (Table 7). These results are confounded somewhat due to the fact that the larger droplets were applied at a higher spray rate. However, the a.i. rates were the same for both nozzle types (0.0168 and 0.014 kg/ha respectively, for Asana-XL and Karate) and the droplet density for the smaller droplets and lower spray rate was almost three times that for the larger droplets and higher spray rate. In a similar study in which microencapsulated and EC formulations of Curacron were applied through the 8002E and 650033 nozzles, Latheef (1991) found that the 8002E nozzle (larger droplets) tended to provide slightly greater mortality of *Heliothis virescens* larvae than did the 650033 nozzle.

Table 7. Effect of droplet size and spray rate on percent mortality of Heliothis virescens neonate larvae and percent undamaged cotton fruit from laboratory spray treatments for two spray nozzle types and two pyrethroids[a].

Nozzle	Spray rate, L/ha	Droplet size, $Dv_{0.5}$, μm	Droplet density no./cm^2	Larval mortality		Undamaged fruit	
				Asana-XL	Karate	Asana-XL	Karate
650033	10.5	180	70	72.2	94.2	77.8	93.6
8002E	21.3	285	25	93.2	98.6	97.0	97.2

[a]After Kirk and House (1992).

Six aerial spray treatments including rotary atomizers, winglets, CP nozzles, and trumpet nozzles were evaluated for efficacy against whitefly (B. tabaci) on cotton (Latheef et al., 1993). Spray droplet density and droplet size for the different treatments were as shown in Table 5. Table 8 shows the reduction in large nymphs following two successive applications of fenpropathin 2.4E + acephate 90S at 0.22 and 0.56 kg (a.i.)/ha, respectively. The interval between treatments was 11 days and the results presented are averages from evaluations 4, 6, 8, and 10 days after each treatment. The trumpet and CP nozzles, which gave the lowest droplet density at the top and mid-canopy heights, generally resulted in the lowest reduction in large nymphs.

Spray Drift

The use of turbine engines on large modern agricultural aircraft has resulted in increased airspeeds while spraying pest control materials. The increased airspeeds, up to 256 km/h (160 mph) in some instances, have resulted in increased concern over the potential effects on spray drift. Our research has shown that increased air shear at spray nozzles due to increased airspeed reduces the Dv.5 of the droplet spectrum and increases the percentage of spray volume in droplets most subject to spray drift (Bouse, 1991). In addition to the spray deposition test previously discussed (Tables 3 and 4), a spray drift test was conducted for the nozzles and airspeeds shown. Spray recovery at different distances downwind and the amount of spray drift collected at 140 m downwind were measured (Table 9). Results indicate that spray drift increased and spray recovery decreased as the airspeed was increased. The smaller droplets produced by the WhirlJet nozzles also resulted in greater downwind drift and lower spray recovery than the larger droplet spectrum sprays produced by the fan nozzles.

Table 8. Mean percentage reduction of *Bemesia tabaci* large nymphs on cotton at top and mid-canopy locations for six spray treatments[a].

Treatment	Top canopy	Mid canopy	Mean
Rotary atomizer 185 km/h, 1.5 m[b]	43.6	50.6	47.1
Rotary atomizer 145 km/h, 1.5 m	53.2	45.8	49.5
Winglets 170 km/h, 1.5 m	43.6	53.7	48.7
CP nozzles 195 km/h, 1.5 m	39.6	52.4	46.0
Trumpet nozzles 175 km/h, 1.5 m	22.6	16.6	19.6
Trumpet nozzles 175 km/h, 3.0 m	22.1	35.5	28.8

[a]After Latheef et al. (1993).
[b]Aircraft speed and height of aircraft wheels above ground.

Table 9. Downwind spray recovery on cotton strings (drift), mylar and WSP cards[a].

Nozzle type[b]	Air speed, km/h	Drift @ 140 m, %	Cumulative recovery @ 140 m, %	Wind speed, m/s
Fan	193	0.8 d[c]	90.4 a	4.1
Fan	217	1.5 cd	74.5 a	3.8
Fan	242	2.6 c	77.9 a	3.7
WJ	193	4.0 b	75.6 a	3.5
WJ	217	4.8 ab	73.4 a	3.6
WJ	242	5.4 a	70.4 a	3.6

[a]After Bouse et al. (1993); WSP (water sensitive paper).
[b]Fan (No. 4020 flat spray) and WJ (1/8B10-8 WhirlJet); Spraying Systems Co.
[c]Means in a column followed by the same letter are not significantly different (P=0.05).

Nonconventional Application

As mentioned earlier, application technology involves the application of both biological agents and conventional pesticides. The Aerial Application Research Unit has been active in development of technology to address nonconventional application problems. Experience has shown that cooperation and interaction among engineers, biologists, entomologists, weed scientists, plant pathologists, and others faced with developing new pest control technology is very important. It is also important that those linkages be formed early in the new technology development process to make sure that delivery systems are considered at the outset. Application technology for new products should be developed at the same time the products are developed so both parts of the system are available and ready for implementation at the same time. In some instances engineers were asked to produce a delivery system after a product was ready for field-scale testing. We have been fortunate on more than one occasion to be able to adapt available equipment to do the job on short notice. Some examples of nonconventional pest control material application efforts that Aerial Application Research Unit engineers have been involved in include corn rootworm attracticides, use of pheromones to control rice borer in sugar cane, use of egg parasites (Trichogramma) to control Heliothis, and use of parasitic mites (Phytoseiulus persimilis) to control two-spotted spider mites in corn.

Table 10. Factors that affect spray deposition, application efficacy, and spray drift.

DEPOSITION	EFFICACY	DRIFT
Spray rate	AI Rate	Droplet size
Droplet size	Spray rate	Air speed
Target size	Coverage	Nozzle
Target shape	Droplet size	Orientation
Spray height	Droplet density	Size
Weather	Formulation	Pressure
Wind	Surface tension	Boom length
Temperature	Absorption	Spray height
Humidity	Translocation	Weather
Formulation	Uptake	Wind
Surface tension		Inversion
Retention		Evaporation
Evaporation		Formulation
Adjuvants		Adjuvants
Canopy density		

Significant progress has been made in understanding of application variables that affect the efficacy and safety of pest control materials. However, deposition, efficacy, and drift control remain important issues that can best be addressed through additional research. A number of variables must be considered in optimizing the aerial application process. A summary list is presented in Table 10. Several of the variables are listed under more than one heading. For instance, droplet size and formulation properties affect the deposition of droplets on targets, the efficacy of materials deposited, and spray drift. Other factors not listed may also be important to the outcome of an application of pest control material.

The "best" solution to optimizing application variables is elusive because test results are not always consistent and, as with the effect of droplet size on efficacy, may vary with the pest and material applied. There remains uncertainty as to the optimization of application variables for a wide range of pest problems. Continuing cooperation between biologists and engineers is essential for development of new technology and its transfer to users.

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Action Area III: Ecology and Population Dynamics

NEW CONCEPTS IN MIGRATION RESEARCH

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The mobility of adult corn earworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), has been noted in pest surveys (Hartstack et al. 1982; Goodenough et al. 1988) and has limited the effectiveness of managing populations on the farm level using cultural, chemical, and biological control (Knipling 1979). Knipling and Stadelbacher (1983) suggested that *Heliothis* populations could be better managed in overwintering habitats before insects migrate and infest larger cropping areas. For example, Hayes and Bell (1994) substantially suppressed early season populations of *H. zea* and *H. virescens* using a single baculovirus treatment over a 238-km^2 area in the Mississippi Delta, but indicated that insect dispersal may have reduced control efficacy. Schneider et al. (1989) released 250,000 internally marked, sterile male, and sterile-male-producing female *H. virescens* over the same area of the Mississippi Delta, and were unable to account for 70% of the males within a 1,500-km^2 area in which the release was centered. Our paper identifies methods for monitoring and predicting long-distance movements of the *H/H* spp. complex, and determining the role of migrants in the population dynamics and ecology in source and recipient areas.

Population Dynamics

Heliothis/Helicoverpa (*H/H*) spp. adults migrate from decaying habitats to locate new larval hosts and adult nectar sources (Fitt 1989). Farrow and Daly (1987) described three arbitrary categories (short-range, long-range, and migratory) of *H/H* spp. movements, each of which contributes to the species' exploitation of host plants over a wide geographic range. Colvin and Gatehouse (1993) found that the absence of carbohydrates from the diet of *Helicoverpa armigera* (Hübner) adults was associated with an increased pre-reproductive period, assumed to be directly related to a propensity for migratory flight. Populations of *H. zea* and *H. virescens* generally migrate en masse from the Lower Rio Grande Valley (LRGV) in response to the relatively synchronized senescence of cultivated hosts such as field corn (Raulston et al. 1986). However, populations of *H. virescens* in the Mississippi Delta tend to increase each generation because the monoculture of cotton remains an excellent larval host (P.D. Lingren, pers. comm.).

Wide area surveys of *H/H* spp. are complicated by the insects' polyphagous nature and wide geographic range. Field surveys of eggs and larvae provide an accurate assessment of the pests' abundance and age distribution, but require a great deal of time to complete. Alternatively, Raulston et al. (1992) conducted pupal excavations and determined that as many as seven billion adult *H. zea* emerged from fields of fruiting corn in the LRGV. Pupal digs can provide a more accurate indication than field surveys of the number and timing of emerging insects before their migration. Networks of grid traps, light traps (Snow et al. 1972), and pheromone traps (Goodenough et al. 1988) have facilitated pest surveys. However, grid traps and light traps are not pest specific, and pheromone traps capture only sexually-active males of a targeted species. Recent advances in the study of adult feeding attractants of *H/H* spp. may soon enhance sampling technologies for males and females of targeted species.

Tracking Migration

Atmospheric Measurements. Vertical profiles of wind velocity and synoptic-scale wind analyses revealed mechanisms for rapid and persistent transport of migrating *H/H* spp. across the Central U.S. Daily atmospheric soundings measured at 0000 Universal Coordinated Time (UTC), 0600 UTC, 1200 UTC and 1800 UTC by the National Weather Service (NWS) documented a wind jet (with maximum values of 28 m/s) at ≈600 m Above

Ground Level (AGL) from southern Texas through central Oklahoma to Iowa (Bonner 1968). The wind jet most frequently attains maximum speed at night when the atmosphere becomes more thermally stable, thereby reducing drag caused by surface roughness. Currently, however, the NWS makes only two atmospheric soundings daily at 0000 UTC and 1200 UTC (approximately local sunset and sunrise, respectively, in the Central U.S.), which may inadequately describe nocturnal winds below 1200 m AGL (Westbrook et al. 1989). Further, the network of NWS atmospheric soundings comprises stations that are ≈400 km apart, making it difficult to overcome topographic and geographic variations in the interpolation of atmospheric data at altitudes below 1000 m AGL.

Increased nocturnal atmospheric measurements in the lowest 2000 m AGL along the flight trajectory will significantly improve the simulation of migratory displacements. Tetrahedral-shaped mylar balloons (tetroons) were tracked to simulate late migratory routes of *H. zea* from the LRGV, eastern Texas, and the Texas High Plains in 1992 (Westbrook et al., in press) and 1993 (J.K. Westbrook, unpublished data). For cases when the tetroon transponder signal was lost prematurely, forward atmospheric trajectories were constructed from wind velocity data obtained by multiple pilot balloons (pibals) launched from successive positions along the approximate atmospheric trajectory; however, the time required for deployment, measurement, and driving (within the posted speed limit) made it difficult for the driver to keep pace with the air parcel displacement. Westbrook et al. (in press) tracked tetroons for ≈400 km per night as markers of *H. zea* migrating from source areas in the LRGV. Tetroon-tracking personnel communicated in real-time with a radar operator to move a mobile, ground-based radar (GBR-2) from the tetroon launch site to a location 200 km downwind at 5 h after launch (Fig. 1). The radar detected the overflight of the tetroon (a surrogate of migrating insects) in the presence of a strongly-veering wind trajectory. In 1993, a pair of tetroons that were launched simultaneously 35 km apart in the LRGV (line A) drifted for 9 h to locations that were 130 km apart (line B) (Fig. 2). The diffluence noted by the polygon connecting the trajectories of 7 June 1993 indicated a potential insect fall-out area of 32,000 km^2 due to winds. Two tetroons launched on 8 June 1993 near (line C) the previous night's trajectory endpoints traveled to locations that were 275 km apart (line D). The polygon connecting the trajectories of 8 June 1993 indicated a potential insect fall-out area of 129,000 km^2 due to winds.

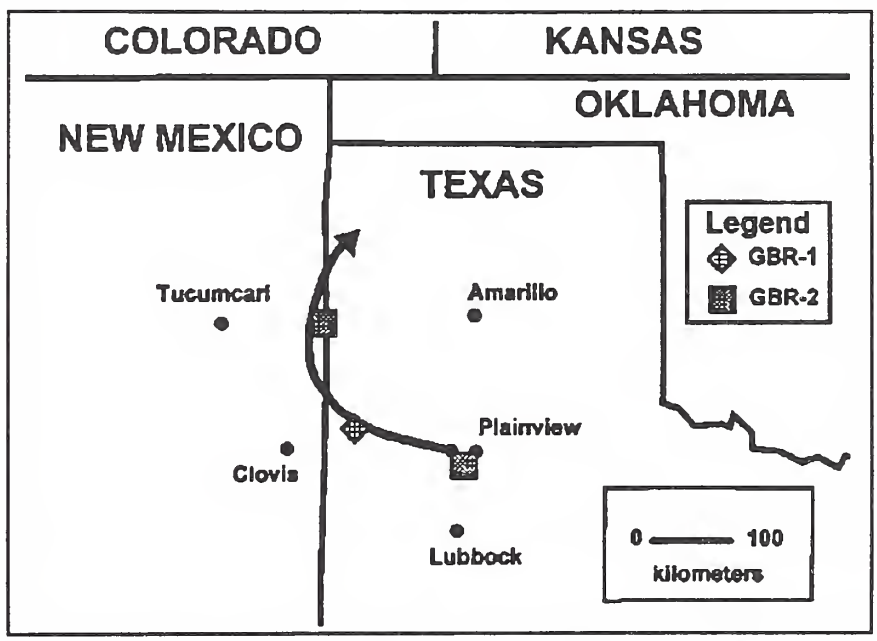

Fig. 1. Location of two ground-based scanning radars (GBR-1 and GBR-2) along a tetroon trajectory from Plainview, TX, on 12 August 1992.

However, actual fallout distributions will be dependent on environmental, physiological, and behavioral factors. Figure 3 shows a vertical cross-section of trajectories of four tetroons launched from Weslaco, TX, in June 1992. The trajectories revealed only minor vertical displacements of the tetroons despite aerial paths over complex terrain. However, no trajectories below 500 m AGL were included. On the other hand, substantial vertical displacements were evident in the vertical cross-section of a tetroon trajectory at 400-600 m AGL over the gradually-sloping High Plains west of Plainview, TX, on 14 August 1992 (Fig. 4). The tetroon trajectory revealed vertical displacements indicative of mesoscale atmospheric turbulence. A line of severe thunderstorms forced the tetroon to the ground at 2.2 h after launch. Local observers reported seeing increased numbers of *H*/*H*-sized moths near the ground.

Figure 5 shows the displacement of a tetroon at 500 m AGL from the High Plains of Texas in advance of an approaching cold front. Remaining ahead (east) of the cold front, the tetroon drifted to north-central Kansas overnight and continued on to northeastern Minnesota 23 h after launch.

Trajectories can also be calculated using measurements from networks of wind-measuring instruments at fixed locations. The U.S.A. is uniquely prepared to measure the lowest 2000 m of the atmosphere using the National Oceanic and Atmospheric Administration (NOAA) 404 MHz Doppler wind profiler (Profiler) network and the tri-departmental (Depts. of Commerce, Defense, and Transportation) scanning Doppler radar (NEXRAD) network. The Profiler network (Fig. 6) comprises ≈30 continuously-operating, vertical-pointing Doppler radars which are located in the central U.S. from Palestine, TX, northward to Wood Lake, MN (NOAA/FSL 1992). Profiler wind velocity data are measured up to 16 km at 250-m intervals, and are disseminated hourly. Wind profilers that operate at 915 MHz are available to measure wind velocity from 100-4000 m altitude, but

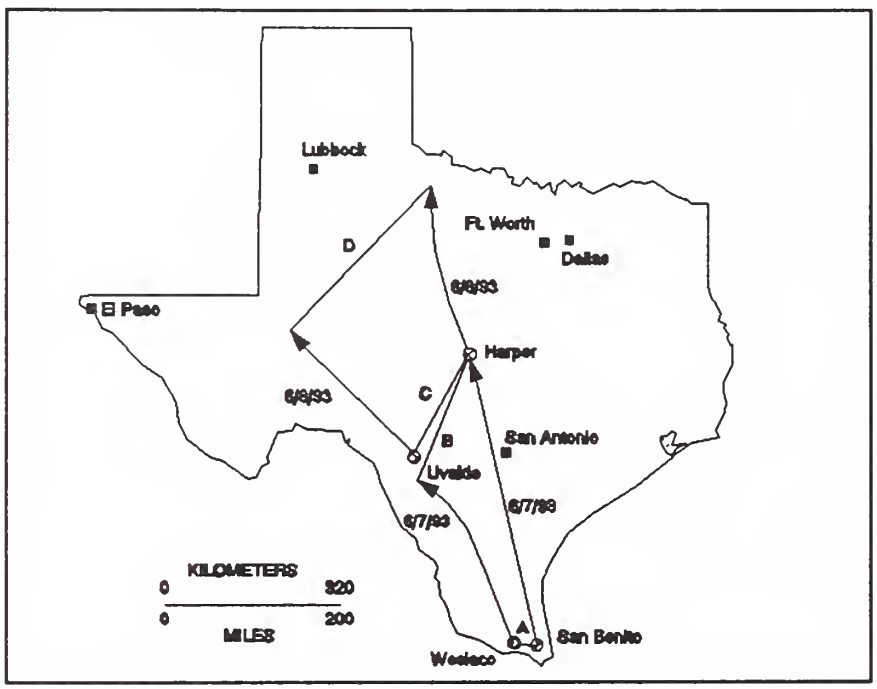

Fig. 2. Paired nocturnal (9-h) tetroon trajectories at ≈500 m Above Ground Level (AGL) in south-central TX on 7-8 June 1993.

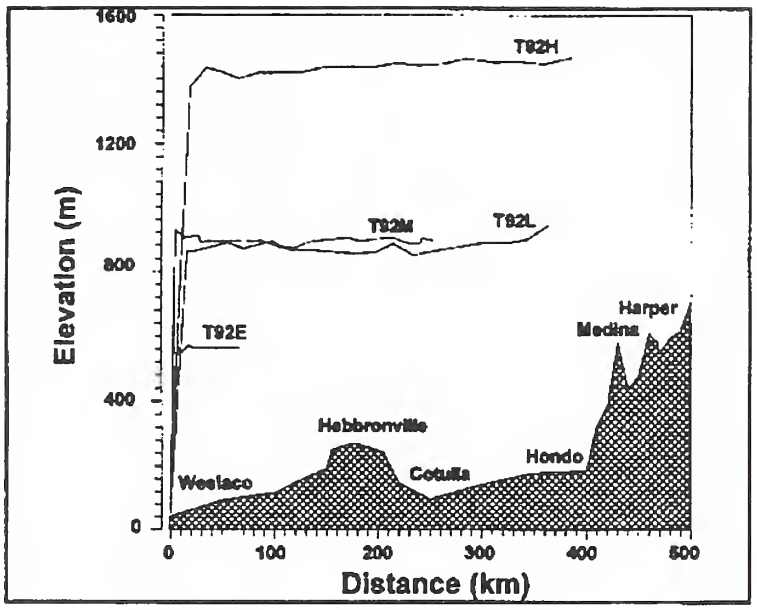

Fig. 3. Vertical cross-section of trajectories of tetroons launched at Weslaco, TX, on 14 June (T92E), 16 June (T92H), 19 June (T92L), and 22 June 1992 (T92M).

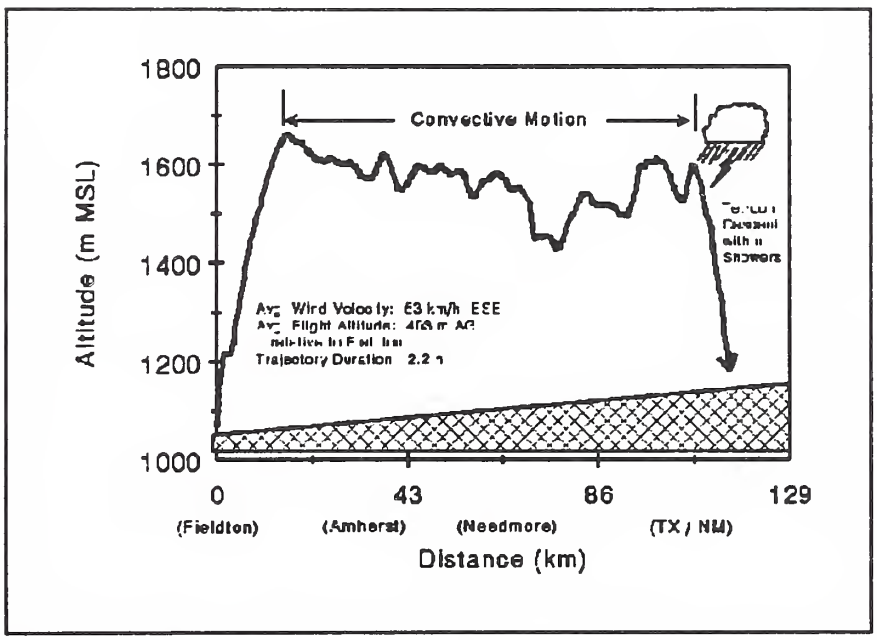

Fig. 4. Vertical cross-section of the trajectory of a tetroon which encountered a line of severe thundershowers 2.2 h after launch from Fieldton, TX, at 0246 (UTC) on 14 August 1992 .

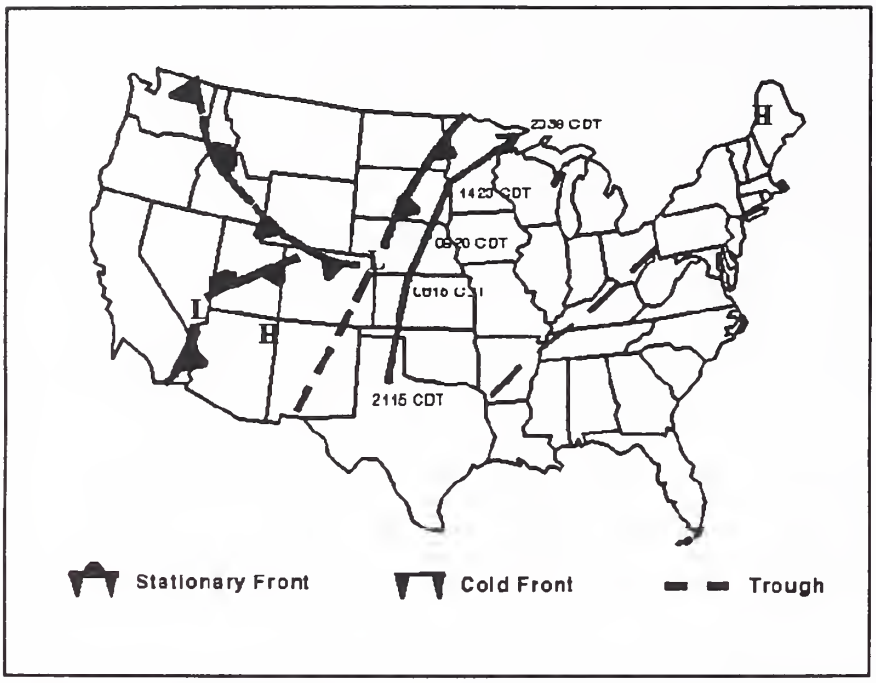

Fig. 5. Argos satellite track of a tetroon drifting at ≈500 m Above Ground Level (AGL) released from Halfway, TX, at 2115 Central Daylight Time (CDT) on 22 August 1992. Synoptic-scale atmospheric features were valid at 0700 CDT on 23 August 1992.

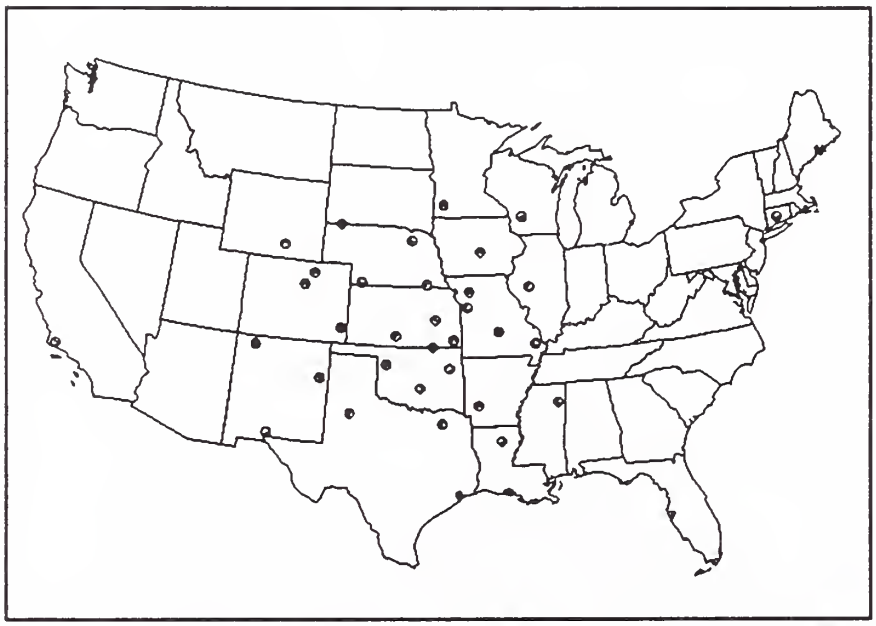

Fig. 6. Distribution of National Oceanic and Atmospheric Administration (NOAA) wind profiler locations in the U.S.A. in 1992.

measurements may be confounded by radar signal returns from insects and birds (Scott A. McLaughlin, pers. comm.). The NEXRAD network of continuously-operating WSR-88D Doppler radars is currently operational at many locations in the U.S., and will include 140 radars in the continental U.S. by 1996 (Crum and Alberty 1993). WSR-88D radars measure target reflectivity and radial velocity of targets with a spatial resolution of 0.25 km to a range of 60 km or a spatial resolution of 1 km to a range of 200 km (see the Remote Sensing sub-section). The WSR-88D radars output vertical profiles and plan position indicator displays of the data every 6 min in precipitation mode and every 10 min in clear-air mode. Volume-averaged wind velocity profiles are disseminated at 0.5-h intervals.

Remote Sensing. X-band radar measurements have documented the occurrence and concentration of insect migrations. These measurements have primarily determined the nocturnal pattern of migration from known source areas of the insects (Wolf et al. 1986). However, the radars have generally remained at fixed locations to monitor overflights. Scanning entomological radars operating at fixed locations can document insect displacements only within a 1.5 km range. Further, the radars document the overflight of *H/H*-sized insects but can not distinguish among species. Two mobile radars located along curved tetroon trajectories on the Texas High Plains detected a mean insect flight speed of 4.5 m/s and mean insect heading 25° counterclockwise to the wind heading (Westbrook et al. 1994). Airborne radar tracked the migratory flight of a cloud of adult corn earworms for 400 km from fruiting corn fields near Weslaco, TX, to San Antonio, TX, in 7.7 h (Wolf et al. 1990). The airborne radar determined the displacement rate of the leading edge of the cloud by repeatedly intercepting the cloud of moths. Alternatively, an array of entomological radars at fixed locations along the migration route could monitor characteristics of overflights.

Scanning entomological radar measurements and radiosonde soundings are being compared with WSR-88D Doppler radar measurements from the NEXRAD facility at League City, TX (W.W.W., J.K.W., and P.D.L., unpublished data). This collaborative effort with the NWS will begin to reveal the accuracy of the NEXRAD Doppler wind velocity measurements in the presence of *H/H* spp.-sized targets, and whether NEXRAD is capable of measuring the concentration of airborne *H/H* spp. The NEXRAD network has tremendous potential for migration research if *H/H* spp. can be detected, especially for continuously surveying the displacements of a migrant population. However, there are presently several confounding traits of the WSR-88D radar products caused by changes in atmospheric refractive index due to radiative cooling, evaporative cooling, and changes of concentration of atmospheric particulates.

Vertical-pointing radars have been used to automatically monitor vertical profiles of insect density and measure radar parameters from which to classify species of migrating insects and characteristics of their flight behavior. Beerwinkle et al. (1993, 1994) described a vertical-pointing radar that was used to automatically measure the annual distribution of insect concentrations from 500 m to 2,300 m AGL. Wolf et al. (1993) found that polarization modulation and radar cross-sections of insects could be compared with their length-to-width ratios to broadly classify insect species. Insect monitoring radars with nutating, vertical-pointing antennae developed in the United Kingdom (Smith et al. 1993) and Australia (Drake et al. 1994) were used to automatically measure the concentration of overflights of insects and obtain information about insects' ground speed, direction of movement, alignment, size, body shape, and migration flux. However, insect monitoring radars require low insect concentrations in order to derive insect flight parameters. Frequency Modulated - Continuous Wave (FM-CW) radar using a fixed, vertical beam has been used to concurrently measure insect concentrations and clear-air atmospheric structure (i.e. changes in atmospheric refractive index which are related to the degree of turbulence) at a spatial resolution of less than 4 m (McLaughlin 1994). Chalon and Johnson (1993) summarized recent developments in airborne radars and lidars, some of which may be able to detect airborne insect targets and fine-scale atmospheric characteristics that affect insect flight behavior.

Aerial Captures. Insect nets have been towed by kites, airplanes, and helicopters, but have rarely captured *H/H* spp. Glick and Noble (1961) flew an airplane equipped with insect-collecting traps for 138.4 h at altitudes from 61-914 m AGL, yet collected only one noctuid specimen: a cabbage looper, (*Trichoplusia ni* [Hübner]).

Beerwinkle et al. (1989) collected 14 noctuid moths of six species -- none within the *H/H* complex -- in a 5-m^2 cross-section net towed by helicopter at altitudes of 75-150 m AGL for 12-15 h. An airplane with two tow nets, each of 0.16-m^2 cross-section, collected five *H. zea* moths (2 male, 2 female, and 1 that was indeterminant due to decomposition in the aerial net) in 0.9 h at 300 m AGL over a large corn field that was a source of emerging *H. zea* near Eagle Lake, TX, in 1991 (K.R. Beerwinkle and P.D.L., unpublished data). However, airplane-towed nets captured no noctuids during flights ($\approx$10 h each) in the LRGV and on the Texas High Plains in 1992 (W.W.W. et al., unpublished data). Twenty-one *H. zea* moths (2.5:1 [male:female]; 100% virgin females) were collected among 110 noctuid specimens collected at altitudes from 60-1,768 m AGL in Texas from $\approx$10 h of flight each year during 1983-1985 (S.D. Pair et al., unpublished data). Callahan et al. (1972) collected a 2-year total of 2,907 *H. zea* adults in ten light traps located between 108 m and 349 m AGL on a tower in southern Georgia. A large air sampling volume per unit time and preservation of captured specimens typically present conflicting requirements for efficient aerial netting of insects.

Mark-recapture. Mark-recapture studies of *H/H* spp. have provided direct evidence of long-distance migratory flights. Snow et al. (1969) monitored the dispersal of natural populations of *H. zea* that had fed on corn injected with the P^{32} isotope with a network of 350 traps on the island of St. Croix (215 km^2, U.S. Virgin Islands). Further, Haile et al. (1975) found that male *H. zea* and *H. virescens* moths migrated 67 km from St. Croix to neighboring islands. Hendricks et al. (1973) found that lab-reared *H. virescens* males marked with dyes dispersed as far as 113 km in 4-5 days in southern Texas. Although geographically-extensive trap networks were necessary to capture adequate samples of synthetically-marked moths for statistical analysis, no known studies deployed traps at distances greater than 113 km from the release sites. Also, lab-reared insects used in many studies may not necessarily have represented the migratory characteristics of the indigenous populations, although Ramaswamy et al. (1985) found no significant difference between the recapture ratios of lab-reared and feral *H. virescens* in pheromone traps at locations from the release site to more than 9.6 km.

Identification of migrants within a population is critical to successful planning of area-wide pest management programs. Progress in the identification of migrants has been made in the area of pollen analysis, especially where exotic pollen such as *Citrus* spp. and *Pithecellobium* spp. naturally mark adults when they feed on the host plant. Exotic pollen found on adult *H/H* spp. have been captured in Oklahoma (Lingren et al. 1993; 1994) and Arkansas (Hendrix et al. 1987), more than 700 km from the nearest source areas of these pollen. DNA analysis of captured *H/H* spp. adults is being investigated as a technique that may pinpoint source areas of the insects, and is discussed by K. Narang elsewhere in this conference report.

Modeling. Atmospheric transport models can be used to estimate long-distance movements of migrating *H/H* spp. adults. Atmospheric trajectory models estimate the displacement rate and direction of an air parcel used as a surrogate marker of a reference point (e.g. center-of-mass or leading edge) for a group of migrating insects. The NWS three-dimensional atmospheric trajectory model uses predicted atmospheric conditions to predict the displacement of air parcels at the surface, the 85 kPa level ($\approx$1,500 m AGL), and the 70 kPa level ($\approx$3,000 m AGL) 24 h in advance and includes changes in the transport altitude caused by forecasted convection and subsidence patterns (Reap 1972). Westbrook et al. (1990) modified the NWS three-dimensional atmospheric trajectory algorithm and calculated atmospheric trajectories as indicators of *H. virescens* migrations at 500 m AGL using wind velocity data measured by the NWS. Atmospheric dispersal models include concentration distributions along trajectories. Westbrook et al. (1992) developed an atmospheric dispersal model that simulated *H/H* spp. flights with passive displacement (no flight velocity) and a 10°C flight threshold temperature. Models that simulate insect flight should include new empirical or theoretical information of dynamic insect flight behavior (e.g. air speed, orientation, vertical distribution of migrants, and flight duration) when it becomes available from the appropriate research areas.

Geographic Information Systems (GIS) are also well suited to migration research because they can present graphical maps of regional distributions of numerous biological and abiotic environmental variables (Liebhold et al. 1993). Additionally, GIS can incorporate and correlate base information about the soil (e.g. type, cover,

moisture, and temperature), climate (e.g. air temperature, absolute humidity, wind speed, wind direction, barometric pressure, and solar radiation), vegetation (e.g. plant types, phenological stages, density, and vitality), insects (e.g. species including parasites and predators, phenological stages, mating status, density, and vitality), pathogens, and non-insect predators. Satellite and aircraft imagery of large areas can be used by GIS to enhance the interpolation of relationships between the pest population and its environment.

Summary

To establish beyond circumstance that migration has occurred, biological and atmospheric mechanisms that contribute to migratory displacements must be measured intensively. Networks of Doppler radars and wind profilers, and moving measurement platforms such as tetroons, mobile ground-based radars, and airborne radars will contribute substantially to advances in migration research. From the intensive biological and meteorological data, relationships between migrant species and their environment can be parameterized. Such information will help determine which migration events impact most significantly crop production within a given area and how large the potential management areas must be. Resulting predictive models should become operational outside the research community.

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Action Area IV: Behavior Modifying Chemicals

RECENT DEVELOPMENTS IN ATTRACTICIDE RESEARCH FOR *HELIOTHIS/HELICOVERPA* SPECIES

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In this paper, the term attracticide is defined as a food bait that is attractive and toxic to a target insect species. In this context, an attracticide is envisaged to be a combination of 1) synthetic volatile chemical mixtures that mimic natural host-plant food attractants, 2) substances which serve as feeding arrestants and/or stimulants, 3) base food materials or carriers, and 4) toxicants concocted in a slow release formulation suitable for field application. Development of effective attracticides for *Heliothis/Helicoverpa* species could lead to new adult management strategies for the corn earworm (*Helicoverpa zea* [Boddie]), tobacco budworm (*Heliothis virescens* [F.]), and other important noctuid pests.

Pesticide control of the *Heliothis/Helicoverpa* species is conventionally directed at the larvae which feed primarily on the fruiting parts of their host plants. Thus, conventional control practices have usually involved high volume broadcast applications of insecticides on a field by field basis. This trend has resulted in major concerns for environmental contamination and food safety among consumers.

Alternative control strategies involving the use of effective attracticides for the highly mobile adults have advantages over conventional practices for managing *Heliothis/Helicoverpa* species. Joyce (1982) estimated that adults were 10-100X more susceptible to insecticides than were larvae, and that adults were much less likely to develop resistance to insecticides. Adult females require a carbohydrate food source, usually obtained from plant nectars, to carry out normal reproductive functions. In addition, early post-emergence feeding on plant exudates and nectars is a high-priority activity for both male and female moths $\leq$ 1 d of age (Lingren et al. 1987, 1988; Beerwinkle et al. 1993). Therefore, the availability of effective attracticides at emergence sites may have good potential for killing adults in their source zones before they can disperse and reproduce in new habitats. The successful development of attracticides and incorporation of this technology into adult management strategies may permit the reduction of corn earworm pest problems over large areas, while substantially reducing the total use of synthetic pesticides and exposure of human foods to pesticide contamination. The development of effective attracticides may also be useful in monitoring populations of noctuid pests.

Various natural and synthetic attractants have been used with some success in attracticide formulations to control and manage other insect pest species. Natural hydrolysates from hydrolyzed proteins of yeasts and corn or soybean bran have been used as attractants in toxic lures for various species of fruit flies (Diptera: Tephritidae) (Steiner 1952, Ayers 1957). Progress of research to develop synthetic attractants that mimic the efficacy of protein hydrolysates for attracting various species of fruit flies has apparently been slow, but Wakabayashi & Cunningham (1991) described a four-component synthetic bait that attracted both sexes of melon flies (*Dacus cucurbitae* Coquillett). Prokopy et al. (1993) recently reported that excrement from various species of birds fed high-protein diets was more attractive to the Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]) than hydrolysates, and they predicted that chemically characterizing the bird excrements could lead to development of improved synthetic attractants for fruit fly pests.

Coppedge et al. (1977, 1978) reported the successful development of an attracticide-based screwworm adult suppression system (SWASS). The SWASS units were composed of a volatile chemical formulation (Swormlure-2) which mimicked the attractiveness of decomposing liver, a food source that stimulated feeding, and an insecticide. The SWASS system was used to suppress the native populations of screwworm, *(Cochliomyia hominivorax* [Coquerel]) adults in areas where sterile males were later released to eradicate the populations.

Considerable research has been conducted to develop attracticide technology for various species of corn rootworms (Metcalf et al. 1987; Metcalf & Lampman 1989; Lance & Sutter 1990, 1991; Hesler & Sutter 1993; Tallamy & Halaweish 1993). Dry granular food baits containing insecticides and natural cucurbitacin feeding stimulants derived from plants of the family Cucurbitaceae have demonstrated effectiveness for controlling several species of corn rootworms. Apparently, cucurbitacins are powerful feeding stimulants for several species of rootworms, but cucurbitacins are not volatile; thus, they are not effective as long-range attractants. It has been hypothesized that the effectiveness of the toxic food baits for rootworms could be greatly enhanced with the addition of volatile attractants (Metcalf et al. 1987). Several volatile chemical compounds have been identified that are differentially attractive to different species of rootworms. For example, the plant volatile constituent eugenol is attractive to the northern corn rootworm (*Diabrotica barberi* Smith & Lawrence) but not to the western corn rootworm (*Diabrotica virgifera virgifera* LeConte); whereas, estragole is attractive to the western corn rootworm but not to the northern species (Ladd et al. 1983, Lampman et al. 1987). Various attracticide formulations for corn rootworms are presently being field tested.

An attracticide formulation composed of a sex pheromone called Grandlure, feeding stimulants, and a toxicant has been developed for the boll weevil, *Anthonomus grandis grandis* Boheman (Smith et al. 1990, McKibben et al. 1990). The formulation is used as a coating for a bait stick which is being field tested as a new, early and late season, control method for boll weevils.

There has been considerable research of the nocturnal behavior, including feeding behavior, of *Heliothis/Helicoverpa* species and other noctuids. Results of nocturnal observations reported by Lingren et al. (1977) indicated that peak feeding times for tobacco budworm adults occurred in early evening and early morning. Adler (1987) reported intensive feeding by corn earworm adults on pigeonpea nectar at dusk in South Carolina. Observations of numerous, apparently newly-emerged corn earworm moths feeding in early evenings on the nectars of various wild flowers, especially *Gaura drummondii* (Spach), growing along the banks of the Rio Grande River during the spring as they moved from corn fields of origin in northern Mexico to breeding habitats in southern Texas were reported by J. R. Raulston (USDA-ARS, Weslaco, TX, personal communication). Haynes et al. (1990) and Heath et al. (1992) identified volatile floral compounds attractive to cabbage loopers (*Trichoplusia ni* [Hübner]) in *Abelia grandiflora* Rehd. and night-blooming jessamine (*Cestrum nocturum* L.), respectively. Landolt et al. (1991) reported the attractance of cabbage loopers to the floral compound phenylacetaldehyde and described an attracticide system for this species which was composed of phenylacetaldehyde, sucrose, and methomyl combined in micropet dispensers. Several researchers (Tingle et al. 1990, Mitchell et al. 1991, Tingle & Mitchell 1992) have reported feeding and oviposition attractance of tobacco budworm adults to volatiles from cotton flowers and other host plants. Beerwinkle et al. (1993) reported observations of intense early-evening feeding activity of corn earworm adults $\leq$ 1 d of age on ergot honeydew on infected dallisgrass growing adjacent to emergence habitats.

Based on extensive nocturnal observations of emergence and early post-emergence behavior which indicated that food seeking is a high priority activity of newly-emerged corn earworm adults, Lingren et al. (1987, 1988, 1989) proposed the use of attracticides as an adult control technique. The viability of this technique was demonstrated by Lingren et al. (1990) who reported major mortalities among newly emerged corn earworm adults that fed on attracticides which had been banded around corn stubble in an emergence habitat.

In 1990, a research program was initiated in the Crop Insect Pests Management Research Unit (CIPMRU) of ARS at College Station, TX, to address the research necessary to implement attracticide control strategies for the corn earworm and other noctuid crop pests. Attracticide development research is a team effort in CIPMRU and team members include an agricultural engineer, a research chemist, and two research entomologists. In addition, the Unit works closely with a research entomologist at the ARS Crop Insects Research Unit at Weslaco, TX, and a research chemist, at Beltsville, MD.

Bioassay Research of Plant Volatile Attractants

Laboratory bioassays have been conducted to determine the feeding attractiveness of several different plant species. Plants selected for testing have been those identified as naturally attractive food hosts by direct observation with the aid of night-vision equipment of adult corn earworm nocturnal feeding behavior or those identified indirectly by the analyses of residual pollen found on proboscises, antennae and other body parts of moths (Lingren et al. 1993, 1994). Additional plants have been selected for bioassay based on their observed attractiveness to other insect species.

Bioassays have been conducted primarily in olfactometers, which were developed for this research. Basic two-choice units which operate on the Y-junction choice principle (McIndoo 1926) were constructed of transparent acrylic plastic materials. Paired volatile baits to be tested were placed in the lower compartments of two cylindrical (8.3-cm ID) bait-sample subchambers (Fig. 1, A & B). Metered, prepurified air was forced (12 liters/min) under positive pressure through each of the two subchambers past the respective bait samples into a

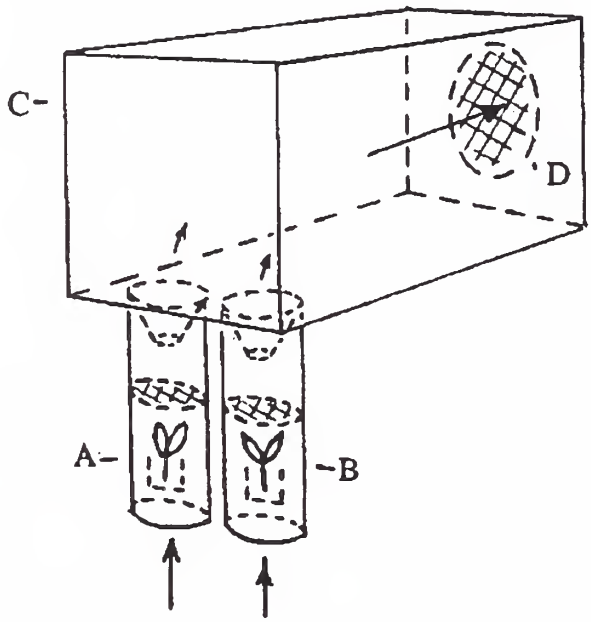

Fig. 1. Schematic of two-choice olfactometer apparatus. (A & B) Bait sample subchambers. (C) Moth-exposure chamber. (D) Filter pad.

large rectangular (23 by 23 by 60 cm) moth-exposure chamber (Fig. 1, C). The supply air with entrained sample volatiles was passed through the large chamber and exhausted through a synthetic-fiber filter pad (Fig. 1, D) which removed moth scales. Air from the filter housing was drawn into a fan-driven exhaust manifold system that maintained a slight negative pressure ($\approx$ 0.40-mm water column) inside the large chamber relative to the ambient external chamber pressure and delivered the air to the outside of the laboratory.

Olfactometer bioassay tests were typically conducted overnight. Approximately 75 moths were placed in each of the large chambers during daylight, the volatile test baits were placed during the dusk simulation period, and the

units were left unattended overnight. Moths seeking one or the other baits during the night, entered a funnel trap over the preferred bait. The next morning at simulated dawn, the moths attracted to the respective baits were counted.

Use of the two-choice chambers with the described procedures permitted evaluation of the relative attractiveness of paired volatile sources. When assaying volatiles from plant bouquets, the cut stems of the plant flowers were placed in a beaker containing a chemical liquid solution (Chrysal solution, Naarden, Holland, The Netherlands) used by florists to keep cut-flowers fresh. In a typical screening test, the attractiveness of volatiles from a plant bouquet was compared to that from a volatile blank composed of a beaker containing only Chrysal solution. Six-choice olfactometer units which operated on the same principles as the two-choice units were also used to determine the relative attractiveness of up to six different volatile baits in direct comparisons. A time-lapse video system (Panasonic Model AG-6720) equipped with a night-vision lens system (Dark Invader, B.E. Meyers & Co., Redmond, WA) was used to monitor moth behavior and time-of-response in the overnight bioassay experiments.

When plant volatile sources of different concentrations were tested against blanks in the two-choice units, the results were typically qualitative, indicating whether or not the plant volatiles were attractive, but failing to indicate a well defined dose/response relationship. In a series of assay tests of *Gaura suffulta* Engelm. bouquets of various sizes (bouquet sizes from 1 to 48 stems with one to three blooms per stem), all assays demonstrated moth attractance to the plants, but there were only slight differences in the comparative moth responses to the plant samples and the respective blanks for the different plant-sample sizes (Fig. 2). However, when two different plant volatile attractants of varying concentrations were compared in the two-choice chambers, dose/response relationships were demonstrated (Fig. 3). Similarly, the moth responses to variable concentrations of volatiles provided by different sized bouquets of the same plant in the six-choice olfactometer units tended to be proportional to the volatile concentrations, indicating clearly defined dose/response relationships (Fig. 4). Thus, the six-choice units have been useful for assaying the relative attractiveness of up to six volatile sources in single tests. Typically, only 30 to 50 percent of the exposed moths responded to the volatile baits in overnight tests with this system.

The feeding attractiveness of volatiles from several different plant sources to corn earworm adults have been demonstrated in two-choice bioassay comparisons of the plant baits to blanks. Examples of results obtained in assays of various plant species follow. The flowering spikes of three different *Gaura* spp. and the honeydew exudates of ergot (*Claviceps paspali* [F. L. Stevens & J. G. Hall]) on dallisgrass (*Paspalum dilatatum* [Poir.]) seed heads were previously observed to be attractive feeding hosts in the field, and the attractiveness of the volatiles from these sources was confirmed in the laboratory (Fig. 5). The three *Gaura* spp. of the family Onagraceae are night blooming plants whose blooms produce fragrant odors and nectar sources that apparently stimulate feeding by corn earworms and other noctuids. In contrast, the dallisgrass ergot honeydew has a musky odor that, to humans, is dissimilar to that from the blooms of the *Gaura* spp.

Pollen from willow (*Salix* spp.) and oak (*Quercus* spp.) were found on corn earworm moths indicating they had fed on those plants (Lingren et al. 1993, 1994). Flowering parts of coyote willow (*Salix exigua* Nutt.), black willow (*Salix nigra* Marsh.), post oak (*Quercus stellata* Wang.), and live oak (*Quercus virginiana* Miller) were tested, and all were found to be attractive to corn earworm adults (Fig. 6).

Blooms on several species of shrubs were observed in the field to be attractive feeding hosts for several species of day-feeding insects. Bouquets of flowering Chinese ligustrum (*Ligustrum sinense* Lour.), Japanese ligustrum (*Ligustrum japonicum* Thunb.), chinaberry (*Melia azedarach* L.), and retama (*Parkinsonia aculeata* L.) were bioassayed in the two-choice olfactometers and found to be variably attractive to corn earworms (Fig. 7). Also, bioassays of some fall-blooming weed species including goatweed (*Croton lindheimeri* Wood), bloodweed (*Ambrosia aptera* DC.), bitterweed (*Helenium tenuifolium* Nutt), and western ragweed (*Ambrosia psilostachya* DC.) indicated they were attractive food sources for corn earworm adults (Fig. 8).

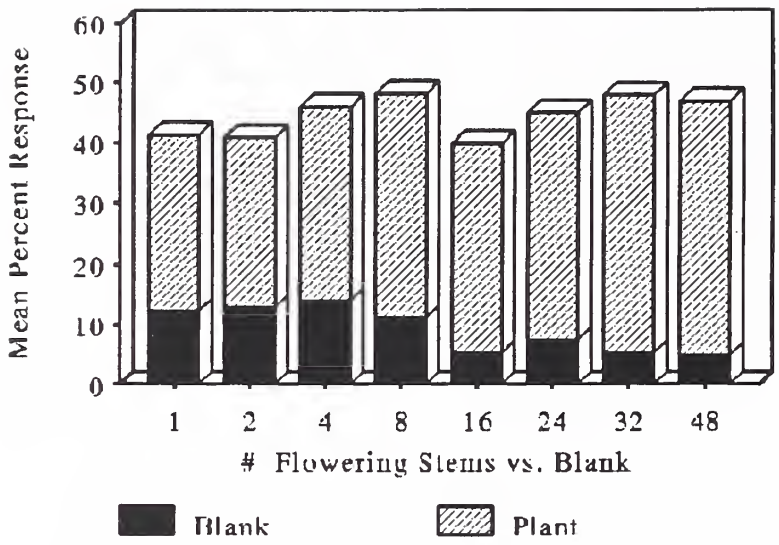

Fig. 2. Mean percent responses of corn earworm adults to volatiles of various concentrations from *G. suffulta* bouquets of different sizes compared to blanks, respectively, in two-choice olfactometers. Moth responses were significantly higher (ANOVA, $\alpha = 0.05$) to plant volatiles than to the blanks in all comparisons, but the correlation of increased response with increased volatile concentrations was poor ($r = 0.39$).

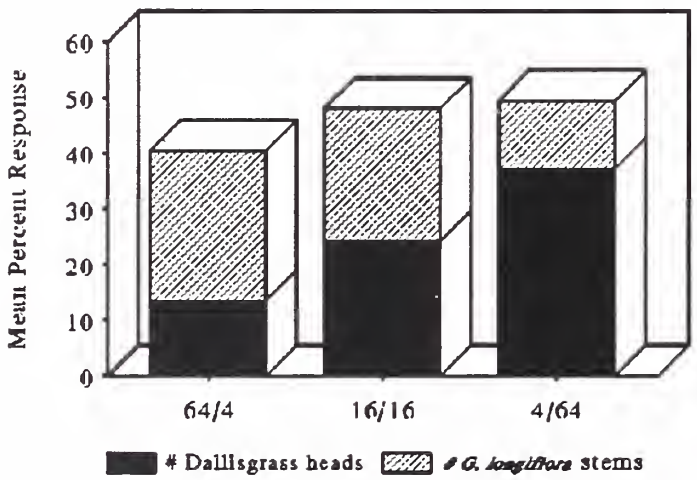

Fig. 3. Dose/response relationship apparent in corn earworm adult attractance to choices of increasing volatile concentrations from dallisgrass ergot versus decreasing concentrations of volatiles from *G. longiflora* bouquets in two-choice olfactometers (No. replications = 8).

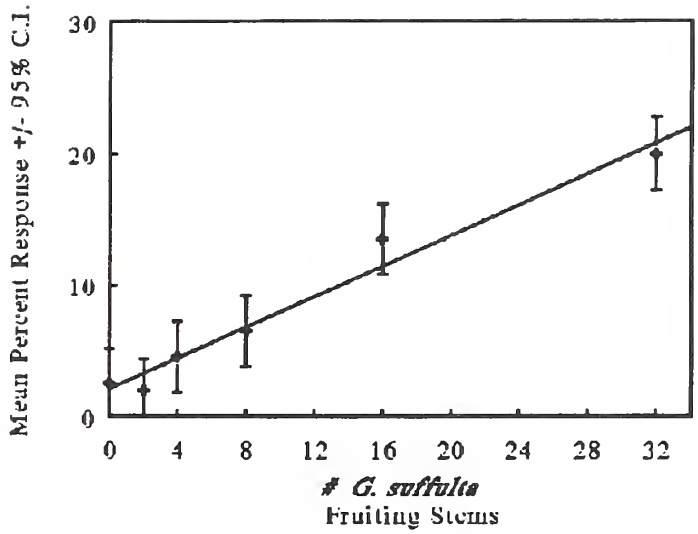

Fig. 4. Dose/response relationship of increased corn earworm adult responses to increased concentrations of *G. suffulta* volatiles in six-choice olfactometer experiments (r = 0.94).

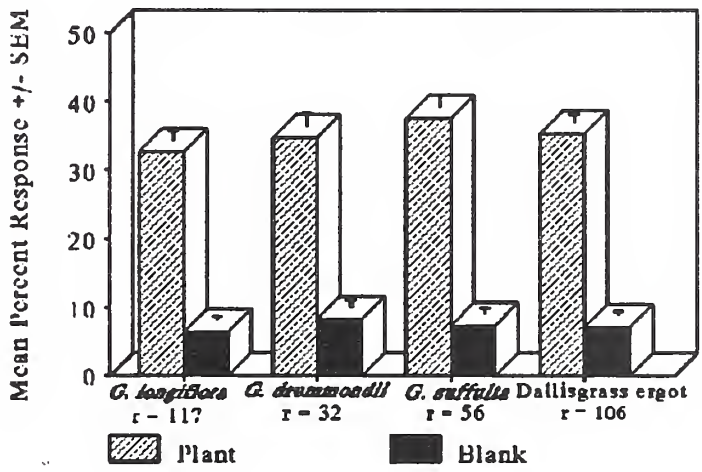

Fig. 5. Mean percent responses of corn earworm adults to volatiles from *G. longiflora*, *G. drummondii*, *G. suffulta*, and dallisgrass ergot bouquets compared with blanks in two-choice olfactometers (r is number of replications for respective comparisons).

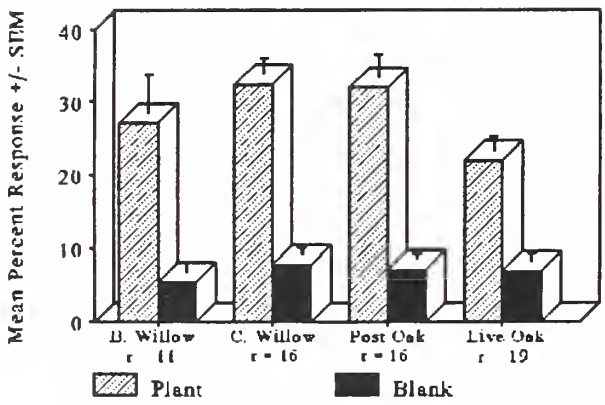

Fig. 6. Mean percent responses of corn earworm adults to volatiles from flowering parts of black willow (*S. nigra*), coyote willow (*S. exigua*), post oak (*Q. stellata*), and live oak (*Q. virginiana*) compared with blanks in two-choice olfactometers.

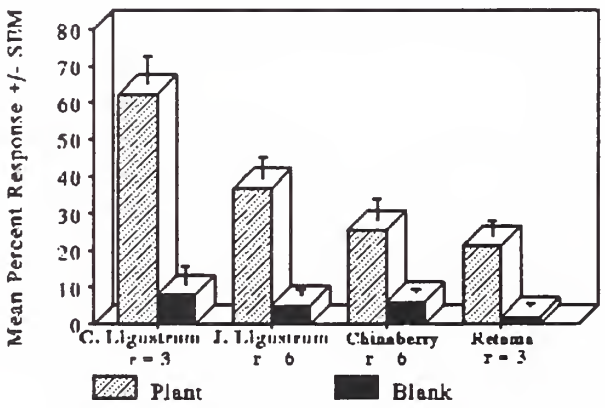

Fig. 7. Mean percent responses of corn earworm adults to volatiles from flowering parts of Chinese ligustrum (*L. sinense*), Japanese ligustrum (*L. japonicum*), chinaberry (*M. azedarach*), and retama (*P. aculeata*) compared with blanks in two-choice olfactometers.

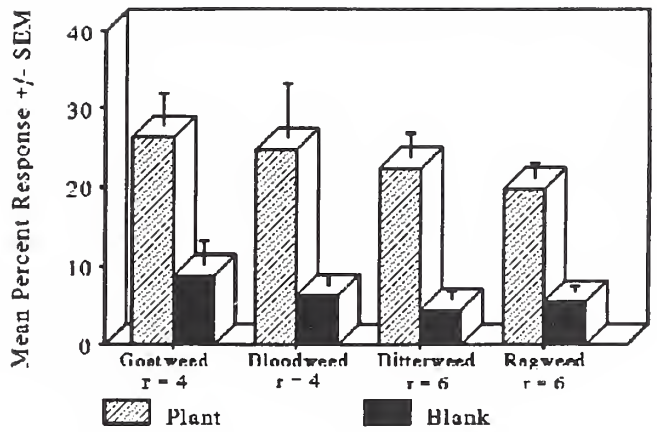

Fig. 8. Mean percent responses of corn earworm adults to volatiles from flowering parts of goatweed (*C. lindheimeri*), bloodweed (*A. aperta*), bitterweed (*H. tenuifolium*), and ragweed (*A.psilostachya*) compared with blanks in two-choice olfactometers.

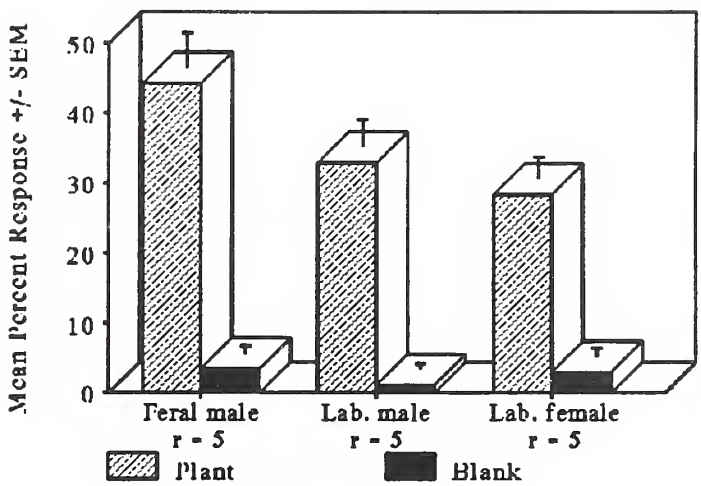

Fig. 9. Comparative responses of feral male, laboratory-reared male, and laboratory-reared female corn earworm adults to volatiles from flowering goldenrod (*S. canadensis*) and blanks in two-choice olfactometers.

Feral male corn earworm moths, field-collected in pheromone-baited cone traps, have been used in the bioassay experiments when they were available. Laboratory-reared males and females from a colony maintained at the Crops Insects Research Unit at Weslaco, TX, have also been used. While feral male moths have usually exhibited greater and more uniform responses in the bioassays than have either the male or female laboratory-reared moths, overall responses of the feral and laboratory-reared moths have been similar. For example, in bioassay tests of goldenrod, *Solidago canadensis* L., the responses of the feral males and the laboratory-reared males and females to the goldenrod baits were all significantly higher (ANOVA, $\alpha = 0.01$) than their corresponding responses to volatile blanks (Fig. 9). While there was an apparent trend for increased positive responses to goldenrod volatiles among the groups (feral males > laboratory males > laboratory females), there were no statistically significant differences (ANOVA, $\alpha = 0.05$) among the positive response levels for the three groups of moths to the goldenrod volatiles. Some variability in the response levels of both the feral and laboratory-reared moths, apparently related to season and short-term weather variations within seasons, has been observed.

The time-of-response during the night of testing, as detected with the night-vision equipped video system, varied somewhat with different volatile sources and different seasons of the year. However, response timing for feral and laboratory-reared males were similar (Fig. 10). Response times for laboratory-reared females has been similar to that of the males. Factors contributing to the observed variability in response timing have not been fully identified and are the subject of continuing research.

Visual appearance is likely a factor contributing to the attractancy of potential food-host plants. Bioassay tests were conducted in two-choice chambers to determine the affects of sight on the attractancy of volatiles from *G. longiflora* bouquets. Similar bouquets of flowering *G. longiflora* were placed in each of the sample subchambers of the two-choice units. One of the bouquets was visible to the moths, and the other was prepared so it was invisible. The results indicated that visibility of the volatile sources was a significant factor (ANOVA, $\alpha = 0.05$), but that the moth responses due solely to olfactory stimuli were substantial (Fig. 11).

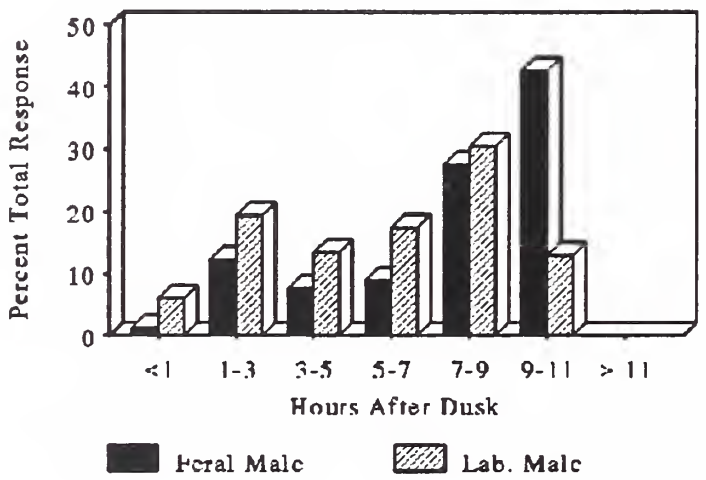

Fig. 10. Time-of-response comparison of feral male and laboratory-reared male corn earworm adults to volatiles from *G. longiflora* blooms in two-choice olfactometers.

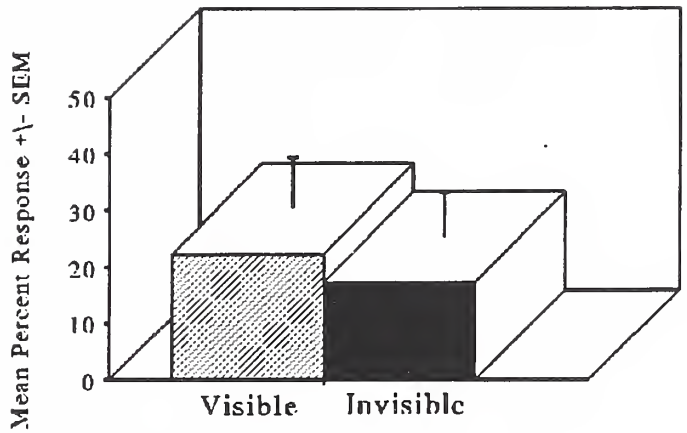

Fig. 11. Effects of volatile-source visibility on olfactometer responses of corn earworm adults to volatiles from *G. longiflora* bouquets. The means were significantly different (ANOVA, $\alpha = 0.05$).

Other Attracticide Development Research

Several different plants that are sources of volatiles, identified in bioassays as attractive to corn earworm adults, have been chemically analyzed (Table 1). The total number of compounds found and identified, or tentatively identified, from hexane extracts of seven different plant sources are listed. Identification of the various compounds was confirmed by comparison to retention times and mass spectra of known standards.

A list of ten identified compounds common to three or more of the seven plant species tested is provided in Table 2. The most striking differences in chemical contents are between the *Gaura* spp. and the other plants. Several compounds are common to goldenrod,bloodweed, and citrus. Of the ten compounds listed, all are common to goldenrod, bloodweed, and citrus except that 2-phenylethanol, linalool and geraniol are absent in goldenrod and bloodweed, methylsalicylate is absent from bloodweed and citrus, and α-humulene is absent from bloodweed. Limonene is present in all plants except *G. longiflora*,γ-terpinene in all plants except *G. drummondii* and *G. longiflora*, and 2-phenylethanol in allplants except goldenrod and bloodweed. Limonene was by far the most predominant compound in goldenrod and bloodweed, while linalool and 2-phenylethanol were predominant in the citrus and *G. suffulta*, respectively. Research is ongoing to identify optimum attractant formulations of these and possibly other chemical compounds.

Proboscis extension response experiments have been conducted in bioassays of numerous natural and synthetic plant materials to identify potential corn earworm adult feeding arrestants and stimulants. Some results are briefly summarized as follows:

1. High stimulatory effect of sucrose was verified.
2. Adults can feed on dry granular sucrose.
3. Field-collected adult corn earworms were less responsive than laboratory-reared moths in comparative tests.
4. Responses of both sexes were similar.
5. Proboscis extension response has proven useful for bioassaying feeding attractiveness of plant nectar sources.

Table 1. Total number of compounds identified in chemical analyses of plants and plant volatiles shown to be attractive to feeding corn earworm adults.

Source Plant	Method of Collection	Total Number of Compounds Identified
Gaura suffulta	Hexane Extract	22
Gaura drummondii	Hexane Extract	12
Gaura longiflora	Hexane Extract	24
Goldenrod	Headspace Volatiles	32*
Bloodweed	Headspace Volatiles	30*
Marrs Orange	Vacuum Volatiles	45*
Rio Red Grapefruit	Vacuum Volatiles	16*

* Some identifications are tentative pending comparisons to authentic compounds.

In research to identify potential toxicants for use in attracticide formulations, toxicity evaluations of certain light-activated dye compounds have indicated that some of the photoactive compounds are effective killing agents for *Heliothis/Helicoverpa* and numerous other insect species. These agents, which must be consumed by the target species, offer a means of adult control with no known detrimental side-effects to humans or the environment. Studies with photoactive compounds are continuing.

Table 2. Selected chemical compounds identified in various plants and plant volatiles that are apparent feeding attractants for corn earworm adults.

Chemical Compound	Source Plant[1]						
	GS	GD	GL	GR	BW	MO	RRG
α-pinene				X	X	X	X
β-myrcene				X	X	X	X
limonene	X	X		X[2]	X[2]	X	X
γ-terpinene	X			X	X	X	X
2-phenylethanol	X[2]	X	X			X	X
linalool						X[2]	X[2]
methylsalicylate		X	X	X			
geraniol	X					X	X
trans-caryophyllene			X	X	X	X	
α-humulene				X		X	X

[1]Plant names: GS - *Gaura suffulta*, GD - *G. drummondii*, GL - *G. longiflora*, GR - goldenrod, BW - bloodweed, MR - Marrs Orange and RRG - Rio Red Grapefruit.
[2]Present in highest level.

Conclusions

Research conducted in the CIPMRU is confirming that corn earworm adults are polyphagous insects as are several other important pest species of Heliothinae (Fitt 1991). However, considerable progress has been made toward the development of attracticides that should be competitive food sources for corn earworm adults in selected habitats. This, coupled with recent advancements in our understanding of aerial transport mechanisms and moth migration behavior (Wolf et al. 1990, Beerwinkle et al. 1994, Westbrook et al. 1994) and the use of natural pollen analyses (Lingren et al. 1994) to identify major moth source areas, makes the future for adult control with attracticide formulations at the moth's habitat-of-origin look promising.

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THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA RIOBRAVIS* AND ITS POTENTIAL AS A BIOLOGICAL CONTROL AGENT FOR *HELICOVERPA ZEA*

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Entomopathogenic nematodes of the genus *Steinernema* are promising biological control agents for a number of insect pests (Kaya 1985, Poinar 1986). These nematodes have a mutualistic association with bacteria of the genus *Xenorhabdus* which are discharged into the hemocoel of their prey by third stage infective juveniles (Akhurst and Boemare 1990). The bacteria kills the host, usually within 48 hours, and provides nutrients for the nematodes to complete their life cycle. Two generations of sexual reproduction occur within the host cadaver, and F_2 progeny, upon reaching the third stage, emerge as infective juveniles which search for new hosts. In the laboratory, steinernematid nematodes are capable of parasitizing an unusually wide range of hosts involving several insect orders. However as Kaya and Gaugler (1993) point out, these laboratory assays eliminate many of the environmental, ecological and behavioral constraints that exist in nature. With soil as their natural habitat the greatest potential for nematodes as biological control agents appears to be against soil inhabiting stages of insect pests (Kaya 1985).

Ecology of Natural Populations of *Steinernema Riobravis* in the Rio Grande Valley

Steinernema riobravis was discovered in the Lower Rio Grande Valley of south Texas and northeast Tamaulipas, Mexico parasitizing prepupae and pupae of corn earworm, *Helicoverpa zea* and fall armyworm, *Spodoptera frugiperda*, in commercial corn fields (Raulston et al. 1992a). About 200,000 ha of corn is planted in the region from late January to early March and it is the largest irrigated corn producing area in the Republic of Mexico (Anonymous 1983). This area is located in the semi-arid subtropics and receives 600-700 mm of rainfall annually. Many soil types exist in the area ranging from 25-70% clay, 15-65% sand, about 15% silt, and with a pH of >8.

Annual surveys involving excavation of corn earworm and fall armyworm from soil in 90-120 commercial fruiting corn fields were conducted in the Lower Rio Grande Valley from 1986-1990 (Raulston et al. 1992b). These surveys showed that 14 to 53% ($\bar{x}$ = 34%) of all fields surveyed contained prepupae or pupae of corn earworm that were parasitized by indigenous populations of *S. riobravis* (Table 1).
Further, from 0 to 36% (average=24%) of surveyed fields contained parasitized fall armyworm. Considering only those fields where nematode parasitization occurred, 20-32% ($\bar{x}$ = 28%) of all corn earworm and 0-84% ($\bar{x}$ = 30%) of the fall armyworm excavated were parasitized (Table 2).

Natural mortality of prepupae and pupae due to all causes ranged from 16-33% ($\bar{x}$ = 24%) for corn earworm and 14-44% ($\bar{x}$ = 20%) for fall armyworm (Table 3). The percentage of all mortality resulting from *S. riobravis* parasitization ranged from 14-68% ($\bar{x}$ = 49%) in corn earworm and from 0-66% ($\bar{x}$ = 46%) in fall armyworm.

These data show a substantial incidence of parasitism of corn earworm and fall armyworm by indigenous populations of *S. riobravis* in the Lower Rio Grande Valley. Considering that most of the fields observed in this study are cropped only once each year and the natural populations are subsequently exposed to extended dry periods and periods with a lack of hosts, the nematode appears to be well adapted to survival under relatively harsh environmental conditions.

Identification of *Steinernema Riobravis* as a New Species

The taxonomic description of *S. riobravis* included morphological, hybridization, and DNA comparisons to other known *Steinernema* species (Cabanillas et al. 1994). For these studies, the nematode was isolated from soil in corn fields located at the Subtropical Agricultural Research Laboratory, Weslaco, Texas. This nematode population was subsequently maintained in the laboratory through *in vivo* rearing on corn earworm prepupae.

Table 1. Percentage of corn fields in the Lower Rio Grande Valley containing corn earworm and fall armyworm parasitized by *Steinernema riobravis*.

	Percent of fields with	
Year	CEW	FAW
1986	52.5	36.4
1987	37.4	21.6
1988	14.1	0.0
1989	28.3	26.6
1990	30.4	11.4
Mean	34.2	24.2

Table 2. Percent of corn earworm and fall armyworm prepupae and pupae parasitized by *Steinernema riobravis* in corn fields in the Lower Rio Grande Valley where at least one parasitized specimen was excavated.

	Percent parasitized[a]	
Year	CEW	FAW
1986	32.1b	37.6bc
1987	45.3a	84.6a
1988	17.9bc	---[b]
1989	19.6c	13.7c
1990	23.8bc	47.6b
	Mean	27.729.5

[a]Column values followed by the same letter are not significantly different as determined by LSD test (alpha = 0.05).
[b]No fall armyworm were collected from samples.

Attempts were made to crossbreed *S. riobravis* with *S. carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. intermedia* by placing individual pre-adult stage males or females of one species with the opposite sex of another species in a

hanging drop of corn earworm hemolymph. For controls, males and females of the same species were suspended in drops of hemolymph. This study indicated no crossbreeding between any of the species tested and progeny were produced only in those crosses involving conspecific males and females (Table 4).

Morphometrically, the overall length and the ratio E (distance from the head to the excretory pore divided by the tail length) can be used to separate *S. riobravis* infective juveniles from *S. carpocapsae*, *S. intermedia*, *S. feltiae* and *S. glaseri*. *S. riobravis* males can be separated from other *Steinernema* species by one or more of the following characteristics: (1) the absence of a terminal tail mucron; (2) strongly curved spicules; (3) the dark golden yellow coloration of the spicules; and (4) the ratio T of the first-generation males (testis reflexion length divided by body length). In addition, DNA sequencing analysis of 304 base pair region of the 26 S ribosomal subunit also showed that *S. riobravis* was different from *S. carpocapsae*, *S. feltiae*, *S. glaseri* and *S. serratum*.

Table 3. Percentage mortality of corn earworm and fall armyworm prepupae and pupae in the Lower Rio Grande Valley resulting from *Steinernema riobravis*.

Year	Total Percent dead		Percent parasitized[a]	
	CEW	FAW	CEW	FAW
1986	29.3	29.6	67.5a	66.1a
1987	32.9	44.2	64.9ab	47.8ab
1988	21.4	19.4	13.5c	0.0c
1989	21.4	14.1	34.7c	23.7c
1990	16.0	14.0	58.3b	35.7b
Mean	23.5	20.1	49.4	46.1

[a]Column values followed by the same letter are not significantly different as determined by LSD test (alpha = 0.05).

Table 4. Results of hybridization experiments between *Steinernema riobravis* and other *Steinernema* species.

	Species *carpocapsae* (All)	*feltiae* (SN)	*glaseri*	*intermedia*	*riobravis*
carpocapsae	+	-	-	-	-
feltiae	-	+	-	-	-
glaseri	-	-	+	-	-
intermedia	-	-	-	+	-
riobravis	-	-	-	-	+

+ = presence of progeny; - = absence of progeny

These characteristics show *S. riobravis* to be a new species. Holotype (male, first-generation) and allotype (female, first-generation) were deposited in the Nematology Collection at the University of California, Davis. Paratypes were deposited in the USDA NC, Beltsville, MD, and in the Laboratories des Vers, Museum National d'Histoire Naturelle, Paris.

Potential of *S. Riobravis* in Area-Wide *Helicoverpa Zea* Suppression Systems

The corn earworm is well adapted to exploitation of ephemeral habitats such as row crop agriculture as a result of its mobility and highly polyphagous behavior (Fitt 1989). These traits facilitate the rapid deployment of populations between fields as well as between crops and naturally occurring host plants. Collectively, these characteristics make it one of the most serious pests of U.S. agriculture. Although highly polyphagous, corn is its preferred host and the presence of this crop usually alleviates pressure on other available hosts (Barber 1937). Indeed, corn acts as a nursery crop which produces large adult populations that, through migration and dispersal, infest other crops on local and regional scales (Raulston et al. 1992b). In the southern U.S., corn is usually one of the earliest spring crops planted and normally produces the populations that infest later-fruiting crops such as cotton. Although field corn is normally heavily infested with corn earworm, control measures are usually not considered cost effective (Pitre et al. 1979). Since larvae feeding on fruiting corn are well protected, survival rates are very high. For instance, corn in the Lower Rio Grande Valley produced from 0.7-3.6 live corn earworm pupae/m^2 between 1985 and 1988 and averaged 2.4 pupae/m^2 (Raulston et al. 1992b) (Table 5).

Estimated corn earworm adult production for the 200,000 ha crop in the Valley ranged from 1.5-7.2 billion for annual adult production of 4.8 billion. If we assume that 10% of this population survives to reproductive maturity, each female lays 1,000 eggs and that 25,000 eggs/ha result in an economic infestation, adults from this region could damage up to 70 times the acreage of the original source area in the F_1. Application of *S. riobravis* to fruiting corn in concentrated source areas would be aimed at that portion of the population which has survived the egg and larval stages, has entered the soil to pupate, and thus is likely to survive to the adult stage. Reduction of this population would effectively reduce the number of adults available for dispersal and thus eliminate or greatly reduce the need to manage migrant corn earworm in crops of migrant recipient zones.

Table 5. Regional production and estimated impact of corn earworm populations produced from corn in the Lower Rio Grande Valley.

Year	Live pupae/ square meter	Estimated adults produced		
		Total (Billions)[a]	Females (Millions)[b]	Potential hectares infested (millions)
1985	3.6	7.2	358	14.3
1986	2.6	5.1	257	10.3
1987	0.7	1.5	74	3.0
1988	2.8	5.5	276	11.0
Mean	2.4	4.8	241	9.7

[a]Assuming 100% emergence
[b]Assuming 10% survival to reproductive stage

Pathogenicity of *S.Riobravis* to Corn Earworm

Laboratory studies. Laboratory experiments were performed at the Subtropical Agricultural Research Laboratory in Weslaco, TX to (1) demonstrate the pathogenicity of *S. riobravis* against corn earworm prepupae; (2) estimate the lethal concentrations; and (3) determine the progeny production of infective juvenile (IJ) nematodes per dead insect in response to nematode concentration (Cabanillas and Raulston (1994b). To demonstrate pathogenicity and determine lethal concentration (LC) values, corn earworm prepupae were placed individually in 60X15 mm petri dishes and exposed to different concentrations (0 to 100) of infective juveniles. The corn earworm were held in the petri dishes for 5 days and all dead prepupae were subsequently dissected to determine the presence of nematodes. Probit analysis (SAS 1988), (Table 6), showed that an increase in the number of nematodes to which the prepupae were exposed resulted in increased in mortality. LC_{50} and LC_{90} values were calculated to be exposure to 13 and 65 IJ/prepupa, respectively. One hundred percent mortality of corn earworm prepupae was achieved in this experiment with exposure to 100 IJ. Comparison of nematodes recovered from infected prepupae to those originally placed in the petri dishes proved that they were the cause of death. Glazer and Navon (1990), reported that 200 IJ of *S. carpocapsae* were required to elicit 100% mortality of *Helicoverpa armigera*.

Production of nematodes per cadaver was also affected by the concentration of nematodes to which they were exposed. The highest average yield of IJ/prepupa (3.75×10^5) occurred at an exposure concentration of 40 IJ/prepupa (Table 7). Regardless of the concentration of IJ's to which prepupae were exposed, the average number of IJ's produced ranged from $2.9\text{-}3.75 \times 10^5$ IJ/prepupae. Average IJ yield from pupae ranged from $1.9\text{-}3.41 \times 10^5$.

Table 6. Effect of different concentrations of *Steinernema riobravis* on mortality of corn earworm prepupae under laboratory bioassay conditions. Data are averages of 5 replicates of 5 corn earworm prepupae each for each nematode concentration.

Nematodes/ prepupa	Percent mortality	Probit value	95% Fiducial limits
1	5	2	1-3
5	20	5	2-7
10	40	10	7-13
-[a]	50	13	9-18
20	55	15	11-21
40	85	48	33-84
80	90	65	43-125
100	100	-	-

[a] The LC_{50} value computed by Probit analysis. The probit regression model is Probit $Y = -2.1 + 1.8 \log_{10} X$ ($P = 0.0001$, $df = 6$, and $Sy.x = 0.078$), where Y = insect mortality response, X = nematode concentration/prepupae.

Table 7. Number of *Steinernema riobravis* nematodes extracted from infected corn earworm prepupae and pupae exposed to different nematode concentrations at 14 days after nematode treatment. A total of 20 prepupae were treated at each nematode concentration.

IJ concentration	Mean number IJ produced (X 1000)	
	Prepupae	Pupae
1	-	-
5	314	190
10	336	288
20	303	296
40	375	341
80	312	304
100	290	305

Greenhouse and field studies. In a greenhouse experiment, corn earworm prepupae were buried in a sterile clay soil held in clay pots at a rate of 10 prepupae/pot. In half of the pots, *S. riobravis* IJ were applied to the soil surface and below surface in the other half. Rates of 0, 70,000, 140,000, 280,000 and 560,000 IJ/m^2 were tested. Five days after treatment, the corn earworm were extracted from the soil, and after an additional 9 days, observed for the presence of nematodes. Data showed that subsurface application of the nematodes resulted in significantly higher parasitism (Table 8). At a rate of 280,000 IJ/m^2 applied subsurface, 90% of the corn earworm were parasitized compared with 46% parasitism with surface application. There was no significant difference in rate of parasitism between the 280,000 and 560,000 IJ/m^2 concentrations; however, a significant reduction was observed between the highest and the lower concentrations.

Table 8. Effects of *Steinernema riobravis* concentration and placement on parasitism of corn earworm prepupae in greenhouse.

Nematode application site	Insect mortality (%) Number of nematodes/m^2 (X 1000)					
	0	70	140	280	560	Mean[a]
Subsurface	0	50	86	90	98	81a
Surface	0	28	32	46	74	45b
Mean	0	39c	59b	68ab	86a	

[a]Row and column means followed by the same letter are not significantly different as determined by LSD test (alpha = 0.05).

To determine efficacy under field conditions, *S. riobravis* IJ's were applied to the soil in plots of fruiting that were infested with corn earworm larvae (Cabanillas and Raulston 1994b). Within each plot, nematodes were applied to 40 single row subplots measuring 1 X 4 m each. Nematode concentrations of 0, 25,000, 50,000, 100,000 and 200,000/m^2 were applied to the plots as follows: (1) when 40% had developed to medium larvae (10-20 mm in length); (2) when 50% had developed to large larvae (>21 mm in length); and (3) when 10% of the corn earworm larvae had exited the ear to pupate. In each plot, six days after 95% of the corn earworm larvae had exited the ears to pupate (12-19 days post treatment), prepupae and pupae were excavated from the middle 2 m^2 of each plot to determine rates of parasitism.

Table 9. Effects of *Steinernema riobravis* concentration and timing of application to soil in corn plots on parasitism of corn earworm prepupae and pupae.

Nematode application time[a]	Insect mortality (%)					
	Nematode concentration (X1000)/m^2					
	0	25	50	100	200	Mean[b]
40% medium larvae	0	29	8	29	21	22b
50% large larvae	14	43	77	92	100	78a
10% cutout	20	68	68	76	95	77a
Mean[b]	11c	47b	51b	66ab	72a	

[a]Nematodes applied when 10% of the corn earworm larvae had exited the corn ear to pupate (cutouts) and when larvae had developed to large or medium size.
[b]Row and column means followed by same letter are not significantly different as determined by LSD test (alpha = 0.05).

There was no significant difference in rate of parasitism when nematodes were applied at 10% cutout compared with 50% large larvae (Table 9). However, a significant reduction in parasitism was noted when nematodes were applied at 40% medium larvae. There was no significant difference in rate of parasitism between application concentrations of 100,000 and 200,000 IJ/m^2, however rates of 25,000 and 50,000 resulted in significantly lower parasitism when compared with the 200,000 rate. When 200,000 IJ's were applied at the 50% large larvae stage, 100% parasitism of prepupae and pupae was attained.

Residual nematode populations were determined from those subplots that received 200,000 IJ/m^2 54-75 days post-application. This was accomplished by obtaining ca. 1 kg of soil from each subplot within each application time. These soil samples were mixed within application time and residual population density was estimated by extracting nematodes from two 100 cc subsamples of the composite soil samples. Corn earworm prepupae were also placed in 100 cc soil samples to determine parasitism rates. Based on extraction of nematodes from the soil samples using the Baermann funnel technique (Baermann 1917) and averaged over application dates, 32% of the nematodes applied to the soil remained 55 to 75 days post application (Table 10). Parasitism rates from laboratory bioassays showed that this residual population effected an average of 85% parasitism of corn earworm prepupae placed in soil subsamples. The residual population may have been augmented by nematodes produced from parasitized corn earworm prepupae and pupae within the plots, however, the magnitude of this possible augmentation is unknown.

In another field test, *S. riobravis* and *S. carpocapsae* (All strain) were compared for their ability to parasitize corn earworm prepupae and pupae (Cabanillas and Raulston, unpublished data). Infective juveniles of both

species were applied to the soil in fruiting corn at rates of 0 to 200,000/m^2. Laboratory reared large corn earworm larvae were contained on the soil surface and allowed to enter the soil for pupation. Prepupae and pupae were excavated after 5 days and observed 7 days post excavation for the presence of nematodes.

Table 10. Determination of residual field populations of *Steinernema riobravis* applied to soil in corn fields and their ability to parasitize corn earworm prepupae in the laboratory.

Number of days post application	Number of nematodes /100 cc soil	Rda (%)	Insect mortality lab bioassay (%)[b]
54	43	22	80
68	90	45	20
75	56	28	90
Mean	63	32	85

[a]Rd is the residual density of nematodes based on the number of nematodes extracted to total number of nematodes applied (200 IJ/100 cc).
[b]Based on 20 corn earworm prepupae exposed/field.

Results (Table 11) showed a significant increase in parasitism by *S. riobravis* between each increase in IJ concentration. The highest rate of parasitism (94.5%) occurred at a rate of 200,000 IJ/m^2. Of interest, the indigenous population resulted in a parasitism rate of 37.3% in the control plots.

The lack of parasitism by *S. carpocapsae* may have been due to the high soil temperatures (>38° C) experienced in this test. These data show that *S. riobravis* is well adapted to high temperatures and is effective at temperatures above the threshold for *S. carpocapsae*. Soil samples were removed from plots receiving 0 and 200,000 IJ/m^2 20 days postapplication to determine residual nematode populations. Results showed that 2 to 3.5 times more *S. riobravis* IJ's were present in plots receiving 200,000/m^2 compared with those where no nematodes were applied (Table 12). No *S. carpocapsae* nematodes were found, again indicating they were unable to survive the conditions of the experiment.

In 1992, *S. riobravis* IJ were applied to fruiting corn plots through in-furrow irrigation to determine the efficacy of this application technique. The nematodes were suspended in water and applied at rates of 0, 100,000, and 200,000/m^2 through a drip system attached immediately in front of the irrigation water source at the head of each row. The irrigation water was applied through gated aluminum irrigation pipes at a rate of 60 liters per min. Each plot consisted of 4 rows of corn measuring 20 m in length and following irrigation, large corn earworm larvae were buried (5 cm deep) in the bottom of the furrow and on the sides and tops of the plant beds. Larvae were spaced at 0.2 m intervals down the entire row lengths in all plots. Larvae were excavated 5 days post nematode application and observed for presence of nematodes. There was no significant difference in the rate of parasitism between the 100,000 and 200,000 rates of application (Table 13). Further, no differences were observed in rates of parasitism between the bottom of the furrow and the side of the bed, however, a significant difference was noted between the bottom and the top of the bed. Under natural conditions, over 95% of the corn earworm pupation occurs on the sides and tops of the plant beds.

Table 11. Comparison of the efficacy of *S. riobravis* and *S. carpocapsae* against corn earworm prepupae and pupae in fruiting corn.

Nematode concentration per m^2 (X 1000)	Percent mortality due to:[a]	
	S. riobravis	*S. carpocapsae*
0	37.3e	0
25	48.5d	0
50	62.0c	0
100	81.0b	0
200	94.5a	0

[a]Means of 4 replications, 10 larvae/replication. Means followed by the same letter within columns are not significantly different as determined by LSD test (alpha = 0.05).

Table 12. Determination of residual populations of *S. riobravis* and *S. carpocapsae* in corn plots 20 days post application.

Number of nematodes applied (X 1000)	Nematodes/50 cc of soil[a]			
	S. riobravis applied		*S. carpocapsae* applied	
	Riobravis	Carpocapsae	Riobravis	Carpocapsae
0	14.2b	0	8.0a	0
200	28.5a	0	9.7a	0

[a]Column means followed by same letter are not significantly different as determined by LSD test (alpha = 0.05).

A similar test was conducted in 1993 in maturing corn plots measuring 4 rows wide by 120 m long. *S. riobravis* IJ's were applied through in-furrow irrigation using the procedures described above, but at concentrations of 0, 25,000, 50,000, 100,000, and 200,000/m^2. At 20 m intervals, 5 corn earworm prepupae were buried at 5 cm in each of the 4 rows in each plot. Prepupae were buried in the plots 6, 15, and 39 days after nematode application. During this period, no rainfall occurred and no additional irrigations were applied.

When prepupae were buried 6 days after nematode application, analyses showed no significant differences in parasitism resulting from location within the field (*viz.* distance from application source in the irrigation water). Averaged over all locations in the field and positions on the row, parasitism ranged from 23% in the control to 97% when 200,000 IJ's were applied (Table 14). There was no significant difference in parasitism rates between applications of 100,000 and 200,000 IJ/m^2. The 200,000 IJ's resulted in a parasitism rate of 97%.

The residual nematode population parasitized 73% of the corn earworm prepupae buried in the plot receiving 200,000 IJ/m^2 15 days post-application (Table 14). Corn earworm parasitism remained significantly higher in the three highest rates than observed in the control 39 days post-application. Averaged over all application rates,

parasitism declined significantly at each time period; however, over 59% parasitism was still noted in the plot receiving 100,000 IJ/m^2 39 days after application. These data indicate the ability of *S. riobravis* to survive extended periods of time in the absence of either hosts or water application.

Averaged over all nematode concentrations, parasitism of corn earworm placed on the side of the plant bed was significantly greater compared to the top of the bed or bottom of the furrow when corn earworm prepupae were buried 6 or 15 days post nematode application (Table 15). However, significantly higher parasitism (81.1%) was noted in the bottom of the furrow when prepupae were buried 39 days post-application. Considering the absence of rain or irrigation after the nematode application, this may have resulted from nematode migration to the bottom of the furrow where the moisture concentration would have been greatest compared with the top or side of the plant bed. Averaged over times, parasitism on the top of the plant bed was significantly lower than observed on the side of the bed or in the furrow.

Table 13. Parasitism of corn earworm larvae by *Steinernema riobravis* applied through in-furrow irrigation.

Distance down furrow (m)	Percent mortality by location on furrow			Percent mortality by distance down furrow
	Bottom	Side	Top	
		Control		
0-5	28.6	12.0	0.0	17.5
5-10	30.0	18.5	0.0	20.3
10-15	33.3	27.3	0.0	26.3
15-20	22.2	23.1	0.0	19.0
Mean mortality	28.9	20.7	0.0	21.1
		100,000/m^2		
0-5	96.9	95.8	90.0	95.5
5-10	94.3	96.7	72.7	91.7
10-15	97.1	92.9	83.3	93.3
15-20	97.1	92.9	93.3	95.4
Mean mortality	96.9	94.2	83.7	93.9
		200,000/m^2		
0-5	100.0	100.0	90.9	98.7
5-10	100.0	100.0	90.0	98.4
10-15	92.0	100.0	100.0	96.8
15-20	100.0	100.0	100.0	100.0
Mean mortality	96.8	100.0	95.5	98.5

Comparison of the Movement Capabilities of *S. Riobravis*

The virulence and ability of *S. riobravis* to move through soil was compared to that of *S. carpocapsae* (Kapow, All, and U.K. strains) and *Heterorhabdita bacteriophora* (Hp88 and H.b. strains) using a modified sand barrier bioassay (Lindegren et al. 1993). A 5.35 cm (inside diameter) X 5 cm long pvc pipe was filled with heat treated sand. Infective juveniles (10/cm^2) were placed on the upper or lower sand surface and 5 fifth-instar greater wax

moth, *Galleria mellonella* larvae were placed on the opposite surface. With this configuration, nematodes are required to move either up or down through the soil column to contact the larvae. Data showed that *S. riobravis* was significantly more virulent than the 3 strains of *S. carpocapsae* regardless of the direction the nematodes were required to move (Table 16). However, there was no significant difference in virulence between *S. riobravis* and the two strains of *H. bacteriophora*. *S. riobravis* was further compared to *S. carpocapsae* (Kapow strain) and *H. bacteriophora* (Hp88 strain) at lower concentrations of IJ nematodes. When nematodes were required to move upward in the soil, *S. riobravis* parasitized over 80% of the wax moth larvae at a concentration of 0.5 IJ/cm^2 compared to ca. 20% and 5% parasitism by Hp88 and Kapow strains, respectively (Table 17). At a concentration of 5 IJ/cm^2, *S. riobravis* resulted in 100% parasitism compared with 83% and 58% for the Hp88 and Kapow strains. Similar results were obtained when the IJ nematodes were required to move downward in the soil. These data indicate that *S. riobravis* is about 20 times more efficacious than *S. carpocapsae* (Kapow strain) and 10 times more efficacious than *H. bacteriophora* (Hp88 strain).

Table 14. Parasitism of corn earworm prepupae following application of *Steinernema riobravis* through in-furrow irrigation. Corn earworm were buried in plots 6 days after application. 1993.

Nematode concentration (X 1000)	Percent parasitism at indicated days after nematode application[a]		
	6	15	39
0	22.5a	29.0a	25.4a
25	66.9b	36.9ab	32.2ab
50	75.1bc	52.3bc	54.4c
100	85.9cd	61.3cd	59.6c
200	96.7d	73.1d	45.1bc
Mean	69.2A	50.5B	43.4C

[a]Means followed by same lower case letter within columns or upper case letter within rows are not significantly different as determined by LSD test (alpha = 0.05).

Table 15. Effect of placement of corn earworm prepupae on plant bed on parasitism by *Steinernema riobravis*. Corn earworm were placed in field six days after nematode application by in-furrow irrigation. 1993.

Row position	Percent parasitism at indicated times post-application (days)[a]			
	6	15	39	Mean
Top of bed	56.9b	17.8c	2.6c	25.5b
Side of bed	82.3a	77.6a	46.1b	68.6a
Bottom of furrow	68.0b	56.2b	81.1a	68.4a

[a] Column means followed by same letter are not significantly different as determined by LSD test (alpha = 0.05).

Effect of Temperature on Parasitism by *S. Riobravis*

Steinernema riobravis was compared to Kapow strain for its ability to parasitize pink bollworm, *Pectinophora gossypiella*, larvae at different temperatures. In this experiment, pink bollworm larvae were exposed to IJ nematodes (15 IJ/larva) at 16, 20, 27, 32 and 38° C. At 16°, 47% mortality occurred in larvae exposed to Kapow, compared to 0% mortality from *S. riobravis* (Table 18). At temperatures of 20 and 27°, Kapow and *S. riobravis* were equally effective resulting in mortality ranging from 91 to 94%. However, at 32°, mortality from Kapow strain decreased to 80% while *S. riobravis* effected 94% mortality. Although Kapow strain was not tested at 38°, *S. riobravis* remained effective at this temperature resulting in 89% mortality.

Table 16. Mortality of greater wax moth larvae exposed to different strains and species of entomopathogenic nematodes. Nematodes (10 IJ/cm^2) were required to move up or down through 5 cm of sand to contact larvae.

Direction of nematode movement	Percent larval mortality[a]					
	S. carpocapsae			*S. riobravis*	*H. bacteriophora*	
	Kapow	All	U.K.		Hp88	H.b.
Down	60.4b	60.4b	25.0c	100a	95.8a	97.9a
Up	35.4b	40.6b	34.4b	100a	100a	93.8a

[a] Row means followed by same letter are not significantly different as determined by LSD test (alpha = 0.05).

Table 17. Mortality of greater wax moth larvae exposed to different concentrations of nematodes. Nematodes were required to move through 5 cm of sand to contact larvae.

Direction of nematode movement	Percent mortality/species/concentration								
	S. riobravis			*S. carpocapsae* (Kapow)			*H. bacteriophora* (Hp88)		
	0.5	1.5	5.0	0.5	1.5	5.0	0.5	1.5	5.0
Down	69.2	79.5	100	1.5	9.2	49.2	17.7	63.1	86.2
Up	83.8	98.5	100	6.9	34.6	58.0	21.5	44.6	83.1

Table 18. Effect of temperature on *S. riobravis* and *S. carpocapsae* (Kapow strain) infection of pink bollworm larvae. Each pink bollworm larvae was exposed to 15 IJ.

Nematode species	Temperature/Percent mortality				
	16	20	27	32	38
S. riobravis	0	94.1	90.6	94.1	89.4
S. carpocapsae	47.0	94.1	92.9	80.0	-

In a similar experiment, the ability of *S. riobravis* to infect pink bollworm larvae at 16, 17, 18, 19, 20, 27, 32, and 38° C was tested (Fig. 1). About 1% parasitism occurred at 16°, however, at 17°, parasitism had increased to ca. 40%, and at 18° to 75%. At 19° parasitism approached 100% and remained above 90% at 38°. The high levels of pink bollworm larval infection at temperatures ranging from 19-38° C is particularly encouraging. Under desert southwest cotton growing conditions, soil surface temperatures may exceed 37° C during midday in early season before plant canopy shading occurs. Thus, high temperature tolerance may be an extremely important nematode characteristic in selecting nematode strains.

Summary

A previously unidentified species of steinernematid nematode was discovered in commercial corn fields in the Lower Rio Grande Valley of south Texas and northeast Tamaulipas, Mexico. Morphometric, crossbreeding, and DNA analysis experiments confirmed the nematode as a new species that has been named *Steinernema riobravis*. The indigenous population resulted in about 50% of the natural mortality occurring in corn earworm and fall armyworm prepupal and pupal populations in the region of discovery.

Laboratory and field tests have confirmed the pathogenicity of the nematode to both corn earworm and fall armyworm. Indeed, application of *S. riobravis* to field plots via spraying the soil or through in-furrow irrigation at rates of 100,000 to 200,000/m^2 has resulted in 90-100% parasitism of corn earworm prepupa and pupae. Comparison of *S. riobravis* to other Steinernematid nematodes under both laboratory and field conditions show that it is more pathogenic against corn earworm, pink bollworm, and greater wax moth. *S. riobravis* also has the ability to withstand higher temperatures than other nematodes to which it has been compared. Research is currently underway to develop technology to utilize the nematode as a primary management tool for suppressing pink bollworm in cotton (Lindegren et al. 1993). Research performed by Biosys Inc. has shown that *S. riobravis* can be efficiently mass propagated *in vitro* with existing technology (Ramon Georgis, personal communication), and that nematode production will not be a constraint in the utilization of *S. riobravis* for controlling a number of pest insects such as citrus weevil, apopka weevil, and mole cricket in a variety of crops.

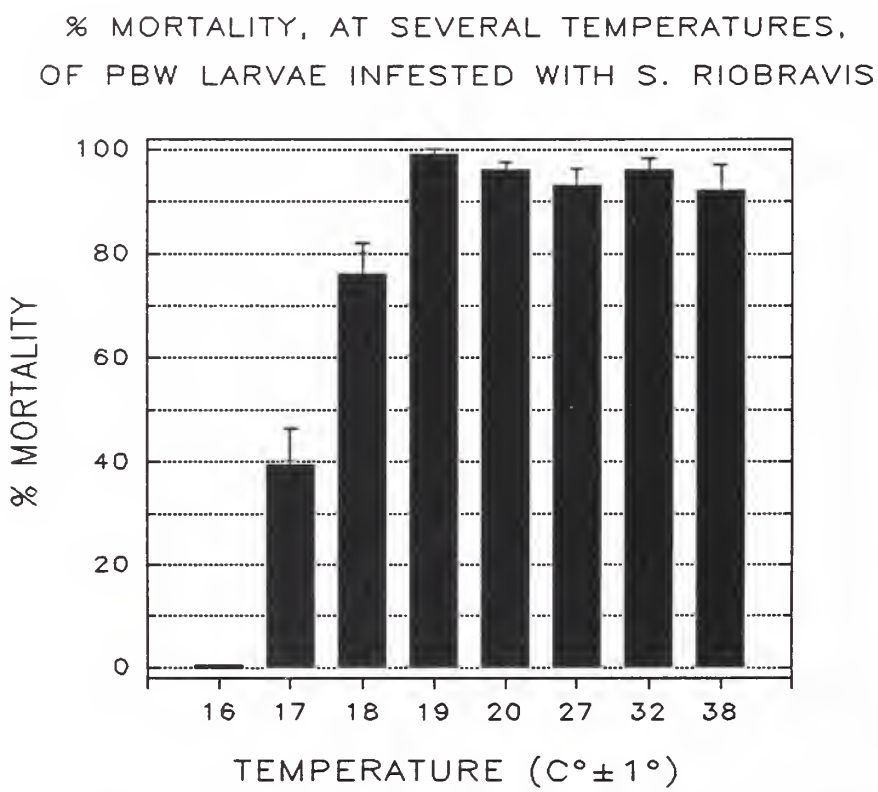

Figure 18. Percentage mortalities of pink bollworm larvae after exposure to 15 infective juvenile *S. riobravis* nematodes/larva at temperatures ranging from 16° to 38°C.

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Action Area VI: Genetics, Molecular Biology, and Basic Physiology

DNA FINGERPRINTING OF *HELICOVERPA ZEA* POPULATIONS: DECIPHERING THE SOURCE OF MIGRANTS

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The cotton bollworm, *Helicoverpa zea* and the tobacco budworm, *Heliothis virescens*, are pests on a variety of crops including cotton, soybean, lettuce, tomato, tobacco, and ornamental crops. The unexpected and sometimes sudden arrival of migrant moths in U.S. cropping systems makes current control efforts ineffective. Therefore, source reduction of pests is crucial for the success of management strategies against these insects.

A major problem growers face every year is that the unexpected and sudden arrival of migrant moths make field-by-field pesticide applications or area-wide control efforts ineffective. Without a preemptive strike to reduce/eliminate the moths at their origins, no control effort is likely to yield any permanent solutions. Source reduction, however, requires that we know the geographical and host-plant source(s) of the immigrant moths.

Though circumstantial evidence supports south (mostly Mexico) to north migration of *H. virescens* and *H. zea*, in the spring and summer, direct markers to identify their origin are lacking. USDA recently initiated an inter-agency effort to research IPM-based area-wide management of these pests. Our research focuses on determination of the origin of migrants by taking advantage of recent advances in DNA fingerprinting technology for the identification of individuals and populations.

Traditionally, allozymes and in a few cases ribosomal DNA (rDNA) RFLPs have been used to study migration patterns (and geographical genetic population structure) in many migratory insects. Some examples include corn leaf aphid, *Rhopalosiphum maidis* (Steiner et al. 1985, Lupoli et al. 1990), cotton leafworm, *Alabama argillacea*, fall armyworm, *Spodoptera frugiperda*, velvetbean caterpillar, and *Anticarsia gemmatalis* (Pashley 1985; Pashley et al. 1985). Seasonal changes in allozyme frequencies and DNA RFLP patterns of resident/overwintering and suspected source populations were used to infer the source of migrants. In insect taxa, such as Hymenoptera and some Lepidoptera (e.g., *H.zea* [Mallet et al. 1993]) which show relatively low levels of allozyme variability, genetic fingerprinting is increasingly used for individual and population marker analysis.

DNA fingerprinting technology (also referred to as DNA typing, identity testing, genotyping or profiling), hereafter referred to as DNA typing involves the use of DNA probes consisting of tandem arrays of short sequences. Generally, two distinct classes of probes are used - the most commonly used probes 'minisatellite' sequences and the 'microsatellite or simple sequence' oligonucleotide repeats. Only recently has DNA typing, which was limited to identification and marker analysis in humans and domestic livestock, been used to analyze differences between wild populations. In addition, organelle DNAs with high mutation rates such as mitochondrial DNA (mtDNA) have been widely used in characterizing genetic population structures (Dowling et al. 1990).

We have initiated DNA fingerprinting studies on *H. zea* to determine the extent and type of genetic differences among geographical populations including resident and suspected source populations. Positive identification of source(s) of migrant moths is the ultimate objective of our research. We have used mtDNA, and clones of rDNA, minisatellite-M13 and repetitive random sequences as probes. We report mtDNA RFLP data documenting genetic differences among populations. We illustrate how statistical and probability theory, when applied to even low-frequency private mtDNA patterns, alone or in combination with other markers (private allozymes and microsatellite DNA fingerprints), can be
used to identify geographic sources of migrant moths.

Sample Collection Sites

Male moths were collected periodically in pheromone traps during the 1991 and 1992 growing seasons from 27 locations in the U.S. with the help of several cooperators. In late April, 1992 collections were made from six sites in Mexico (Drs. J. Leora and J. Lopez) as shown in Figure 1. Samples of resident populations (adults reared from overwintering pupae) were collected at Tifton, GA (November 16 and December 6, 1993) and Mountain City, GA, approximately 380 km north of Tifton (Collections on November 20 and 23, 1993).

Figure 1. Collection sites of *H. zea* and *H. virescens* in Mexico and U.S.A. Names of sites in Mexico (1-6) are shown on the map. 7-12 Texas (7, Weslaco; 8, Hidalgo; 9, Wharton County; 10, Bell County; 11, College Station, and 12 Lubbock); 13, Brawley, CA; 14, Yuma, AZ; 15, Anthony and 16, Artesia, NM; 17, Bossier City and 18, Winnsboro, LA; 19, Quincy, FL; 20, Tifton, GA; 21, Stoneville, MS; 22, Altus and 23, Lane, OK; 24, Desha-Drew and 25, McGeehee, AR; 26, Jackson and 27, Knoxville, TN; 28, Oxford, NC; 29, Manhattan, KS; 30, Columbia, MO; 31, Columbus, OH; 32, Ankeny, IA; 33, Corvalis, OR; 34, Mountain City, GA.

DNA Fingerprinting Approach

Choice of DNA Typing Probes: Ideally, species-specific clones of hypervariable sequences must be isolated for use as DNA typing probes. This requires some prior knowledge about the organization and complexity of the genome of the species under study. The nuclear genome of eukaryotes is extremely large and complex with the relative proportions of single copy and repetitive DNA varying greatly among species. Single-copy DNA includes most of the protein coding genes and some non-coding intergenic sequences. The repetitive DNA includes coding sequences for gene families such as ribosomal RNA and noncoding sequences such as satellite DNA. Only during the past decade has considerable progress has been made in analyzing coding regions and variable noncoding regions of the nuclear DNA in economically important insects. Consequently, the sequence variation in the mtDNA has been most widely used as intra- and interspecies markers in a wide variety of taxa, including *H. zea* (Narang et al. 1994).

In DNA typing studies, short tandemly repeated sequences are preferred because of their high copy number and the relative ease of assay. Mutations in hypervariable regions (HVR) can result in a variable number of tandem repeat (VNTR) alleles. On the other hand some dispersed repeat families of hypervariable sequences, operationally equivalent to single copy genes in their detection by transfer hybridization if used as probes enable variation to be assayed at a large number of loci simultaneously. In addition, animal mtDNA, which as a general rule, evolves faster than genomic DNA can be used for individual and population marker analysis (Simon, 1991; Dowling et al. 1990). It is best to use probes from the same species one is analyzing (homologous probes) but this is not always possible. In many insect species, including *H. zea* and *H. virescens*, such homologous probes are not available. If homologous probes are not available many multiple-copy heterologous probes can be used to study variability in another species under reduced stringencies for both filter hybridization and washing.

At present, knowledge concerning the organization and the complexity of the pest Lepidoptera genomes is completely lacking. Recently we have initiated studies on cloning and DNA sequencing of hypervariable sequences (microsatellite and minisatellite). DNA sequencing of putative clones is in progress. Therefore, during the present studies on *H. zea* populations, we used the following four probes: (1) species-specific mtDNA (Narang et al. 1994); (2) heterologous nuclear ribosomal DNA from *Anopheles quadrimaculatus* (Courtesy of Sharon Mitchell, USDA, ARS, Gainesville, FL); (3) the bacteriophage M13; and (4) a set of four clones of random repetitive sequences of *H. zea* (unpublished data).

1. MtDNA Probe: In addition to occurring in multiple copies (500-5000 per cell in mammals), there are many reasons that make mtDNA suitable for examining the genetic makeup of populations and their history (Tegelstrom, 1992, White and Densmore, 1992). These include: (1) maternal-haploid inheritance, (2) small size as compared to the nuclear genome (15-16 Kb in most animals) (3) relatively rapid rate of evolutionary change as evidenced by extensive intraspecific variation in most species studied so far, (4) general conservation of gene order and composition, (5) relative ease of isolation and purification, and (6) availability of primers against conserved regions of many genes of mitochondria.

There is one major drawback to using mtDNA for population-level comparisons--lack of recombination makes mtDNA RFLP haplotypes equivalent to multiple alleles at a single locus. Consequently, gene diversity estimates have larger standard errors than those expected from analysis of nuclear loci. Levels of differentiation between populations tend to be significantly higher than those within populations thus allowing a qualitative assessment of the genetic makeup of species (review in Dowling et al. 1990).

The techniques used for the analysis of mtDNA RFLP variation in *H. zea* (isolation of pure mtDNA, individual's total cellular DNA, restriction digestion, DNA gel electrophoresis, southern blot hybridization, autoradiography and scoring of RFLP patterns) were the same as described by Narang et al. (1994). We stripped and reprobed the transfer blots according to the method described in Kirby (1992)

2. rDNA Probe: Genes coding for ribosomal RNA (28s, 18s and 5.8s) occur in tandem repeat clusters in most species. The tandem repeat unit in all species studied to date consists of (1) an external transcribed spacer, ETS (leader promoter region), (2) an internal noncoding transcribed spacer region, ITS, (3) coding regions for 28s, 18s and 5.8s rRNA separated by ITS and (4) an intergenic non-transcribed spacer segment (NTS). Different parts of the repeat unit evolve at different rates, but the most variable part of the rDNA repeat, the noncoding intergenic spacer sequence reveals high evolutionary rates. Therefore, variation in the spacer sequence has been widely used in DNA typing of insect intraspecific population structure (Post et al. 1992) We used a heterologous clone of the rDNA gene repeat of *Anopheles quadrimaculatus* cloned into the plasmid pUC19.

3. Repetitive DNA Probe--M13 Sequence: The M13 hypervariable region is among the most common multilocus probes used to detect large sets of independently segregating loci in a number of species (review by Bruford et al. 1992). This bacteriophage contains two clusters of 15-bp repeats in the protein II gene, which hybridizes with homologous eukaryotic sequences.

4A. Repetitive DNA Probe--Microsatellite Sequences: Microsatellite DNA regions are short repetitive segments of varying lengths of di or trinucleotides repeats such as $(dG\text{-}dT)_n$ or $(dC\text{-}dA\text{-}dG)_n$ widely dispersed throughout the genome of most species Because of their high copy number and dispersal in the genome, the clones containing di or trinucleotides along with flanking unique sequence DNA can be used for analysis of population-level variation. In addition, primers against unique flanking regions can also be used to amplify microsatellite loci by the polymerase chain reaction (PCR) to detect copy number variation (Hoelzel and Green 1992).

4B. Repetitive DNA Probe--Minisatellite Sequences: Minisatellite sequences consist of tandem arrays of up to several hundred 15-60 base pair units, often widely dispersed throughout the genome of almost all species. The tandem repeat units of different minisatellite loci share a common 'core' sequence. Several different core sequences have been identified, which can be used to detect polymorphism at large number of loci in many different species. Generally, minisatellite (multilocus DNA) probes are not suitable for population variability studies, because they yield too many DNA bands (fingerprints) for meaningful analysis (which band belongs to which locus). For population-level comparisons, use of a set of hypervariable single-locus nuclear probes makes the identification of specific loci and alleles straightforward. Allele frequency differences in wild populations can be easily analyzed. In addition, organelle DNA with high mutation rates, such as mtDNA has been widely used as genetic markers in characterizing genetic population structures.

4C. Repetitive Anonymous DNA: We used a set of four clones of random repetitive sequences of *H. zea* (unpublished data): (1) (AT)5 type; (2) GAA type; (3) AAG type; and (4) polypyrimidine rich type.

Interpretation of RFLP Profile Matches: The ability to match a putative migrant to its source population can be influenced by many factors. One common factor is the possibility of having skewed mtDNA haplotype frequencies resulting from analysis of a small sample size from the wild populations. The statistical power of the DNA identity analysis is based on the low probability that different individuals or populations share the rare or private haplotypes (or alleles at a number of loci) by chance. We have therefore used probability and statistical analysis of mtDNA haplotype frequency data.

Statistical and Probability Analysis: The methods of probability and statistical analysis of the population DNA fingerprinting data of Kirby (1992) were used. Natural populations of H. zea differed in mtDNA pattern and haplotype (and allozymes-unpublished data) frequencies. The confidence intervals for a population proportion π of frequencies were calculated by the following formula:

$$p - z_{a/2}\sqrt{\frac{p(1-p)}{n}} \leq \pi \leq p + z_{a/2}\sqrt{\frac{p(1-p)}{n}}$$

The quantity $z_{a/2}$ is the value a standard normal random variable exceeds with probability a/2. For example, for 90% confidence intervals, $z_{.05} = 1.645$ and for 95% confidence interval, $z_{.025} = 1.96$. The p and n refer to frequency of characteristic DNA fingerprints and population sample size respectively. This formula is applicable to population π proportion when the sample is sufficiently large that np and n(1-p) both exceed 5.

The ultimate question that must be addressed is: how can one determine whether a given moth caught in a trap is a migrant or a resident moth. To answer this, one would need to have a comprehensive knowledge of the genetic make-up of U.S. resident populations (from overwintering populations), monitor changes in the genetic structure during the moth growing season, identify new patterns and their frequencies, and then identify the possible origin of migrants from the suspected source (for example, Mexico). So far we have analyzed samples of resident moths from Georgia (2 sites) and compared them with moths collected in pheromone traps at three sites each in the U.S. and in Mexico. To illustrate the RFLP analysis data, we have used mtDNA haplotypes of samples from one site in U.S. and one from Mexico. We used the empirical additive probability theory; i.e., the probability that one of the several independent events occurs is the sum of the probability that the individual events occur as shown by the formula:

P(A or B or C ...) = P(A) + P(B) + P(C) + ...

In other words, the probability that an individual moth in a U.S. cropping site will have any one of the Mexican-specific mtDNA haplotypes is the sum of their probabilities (frequencies) in Mexican population.

Results

Mitochondrial Restriction Patterns: Initially 41 restriction enzymes (REs) including 6-bp cutters and 4-bp cutters were used to cleave DNA of individual moths for the mtDNA RFLP analysis (see Narang et al. 1994). Five of these enzymes (Dra I, EcoRV, Nde I, Nsi I and Rsa I) have A-T rich recognition sites. Of the forty-one, only 15 enzymes (Table 1) that produced polymorphic patterns in one or more populations were selected for further analysis. The southern blots probed with radiolabeled mtDNA were reprobed with the rDNA probe and in some cases with satellite sequences. Ideally, for rDNA, it is desirable to use REs with recognition sequences in the NTS region of the rDNA repeat unit. However, we have not yet cloned and mapped restriction sites of rDNA repeat in moths. Since the NTS in most insect taxa are so variable it is quite likely that the restriction enzymes cutting only in the NTS (i.e. NTS repeats) of *H. zea* would not be detected by the mosquito DNA probe. Therefore, experiments are being conducted to determine whether some of these fifteen REs (used for mtDNA RFLP analysis) have restriction sites within the variable region.

Mitochondrial Restriction Pattern Differences Among Populations: Table 1 shows the number of restriction patterns observed from the analysis of mtDNA of individual adults with several enzymes. Of the forty-one enzymes used, eighteen (44%) had either a single site or no site. Eight (19.5 %) produced (monomorphic patterns). Fifteen (36.5%) restriction enzymes yielded 2 or more (polymorphic) patterns. Thirteen of these enzymes produced polymorphic patterns at the 95% criterion, i.e., the frequency of the most common pattern is 0.95 or less. The other group of 2 enzymes produced polymorphic patterns only at the 99% criterion, i.e., the most common pattern has a frequency of 0.99 or less. These 2 produce patterns in which the frequency of most common pattern was between 0.96 and 0.99.

The number of restriction patterns in the 8 populations ranged from 2 to 12. These included 2 with Hpa I, 3 with Dra I, 4 with Ase I Mbo II, Sau96 I, and Sca I, 5 with Hpa II/Msp I, 6 with EcoR I, and Hind III, 7 with Rsa I, 8 with Hinf I, 9 with Alu I, and 12 with Mbo I/Sau3A I.

Table 1 also shows mtDNA RFLP patterns in *H. zea* populations. Of the 74 polymorphic patterns, 17 (pattern A of each of the 13 restriction enzyme, C of Mbo, B of Alu I, Hpa I and Hpa II/Msp I) were observed in 7 or all 8 populations. A majority of patterns (59.5%) occurred in 1 or 2 populations. These include 35-single

population and 10 two-population patterns (Table 1). The single-population patterns include: Alu I-C, D, G, H, I; Ase I-C, E, ; Dra I-C; EcoR I-C, E, F; Hind III-B, D, E, F; Hinf I-B, D, E, F, H; Hpa II-E; Mbo I-D, G, I, J, K; Mbo II-B, D; Rsa I-D, F, G; Sau96 I-C, D; and Sca I-B, D. The two-population patterns include: Alu I-E; Ase I-B; Hinf I-G; Hpa II-C, D; Mbo I-E, L; Rsa I-B, E; and Sca I-C. The three-population patterns include: Alu I-F; EcoR I-D; Hind III-C; Mbo I-C, H; Rsa I-C and Sau96 I-B. The four-population patterns include: Dra I-B; EcoR I-B; Hinf I-C; and Mbo I-B. Only one pattern, Mbo I-F was present in 6 populations. In addition to differences in frequencies of certain patterns, moth collections from Mexico and U.S. differed in 43 diagnostic patterns. Of these 16 were found only in Mexico and the other 27 only in the U.S. Of these 43 patterns, 35 (81%) were private in that they were observed only in single populations (22 in U.S. and 13 in Mexico).

Mitochondrial Haplotype Differences Among Populations: Table 2 shows the flexible scheme used, to simplify the identification and designation of mtDNA restriction haplotypes. Patterns of an individual moth based on eight restriction enzymes (Hpa I, Hpa II/Msp I, Mbo I/Sau3A I, Mbo II, Rsa I, and Sau96 I) can be assigned to one of the seven major 1-digit haplotype categories. Further classification of haplotypes (2-digit designations), such as 1.1, 1.2, 1.3, etc. (shown in Table 3) are based on numbers of different restriction patterns resulting from more than one enzyme including those indicated by "V" in Table 2, and others, such as - Ase I, Dra I, EcoR I, Hind III, Hinf I, and Sca I. When necessary, further differentiation among the 2-digit haplotypes can be accomplished by using 3-digit designations based on Alu I patterns (as shown in Table 5). The Alu I - A, B, C, D, E, F, G, H and I are shown as 1, 2, 3, 4, 5, 6, 7, 8 and 9 respectively as third digits in the 3-digit haplotypes (Table 3).

Table 3 shows a list of 47 different 2-digit haplotypes. These included 11 in Category-1, 10 in Cat-2, 8 in Cat-3, 5 in Cat-4, 3 in Cat-5, 2 in Cat-6, and 8 in Cat-7. When we included patterns of Alu I, more than 60 3-digit haplotypes were identified (data not included).

Table 4 summarizes the frequency distribution of haplotypes in two populations, one representing the resident population from Tifton, GA and the other from Zaragosa, Mexico. The objective is to determine the potential diagnostic (probability) values of population-specific haplotypes for identification of migrant moths. Of the 30 diagnostic haplotypes between the two populations, 16 were observed only in samples of resident moths from Tifton and 14 in trapped adults from Zaragosa (Table 5).

Table 1. Frequency distribution of mtDNA RFLP patterns in populations of *H. zea* from Mexico and U.S.A.[a]

Restriction patterns		POPULATIONS							
		U.S.A.					Mexico		
		COR	ANK	WES	TIF[b]	MOT[b]	ZAR	HER	DEL
Alu I	*n*	29	45	41	34	26	35	33	39
	A	0.55	0.58	0.44	0.29	0.58	0.46	0.73	0.46
	B	0.45	0.38	0.49	0.68	0.42	0.34	0.21	0.51
	C	0.00	0.00	0.00	0.00	0.00	0.03[c]	0.00	0.00
	D	0.00	0.00	0.00	0.00	0.00	0.03[c]	0.00	0.00
	E	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.03
	F	0.00	0.00	0.07	0.00	0.00	0.06	0.06	0.00
	G	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	H	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	I	0.00	0.00	0.00	0.03[c]	0.00			
Ase I	*n*	24	46	45	34	26	36	40	42
	A	0.96	1.00	1.00	1.00	0.92	1.00	0.97	1.00
	B	0.0	0.00	0.00	0.00	0.04	0.00	0.03	0.00
	C	0.04[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	E	0.00	0.00	0.00	0.00	0.04[c]	0.00	0.00	0.00
Dra I	*n*	42	55	56	34	26	42	48	47
	A	1.00	1.00	1.00	0.97	0.92	0.98	0.98	0.96
	B	0.00	0.00	0.00	0.03	0.08	0.02	0.02	0.02
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02[c]
EcoR I	*n*	36	33	47	34	26	30	40	44
	A	0.97	0.97	0.89	0.79	1.00	0.97	0.95	0.91
	B	0.00	0.00	0.11	0.09	0.00	0.03	0.025	0.00
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.025[c]	0.00
	D	0.03	0.03	0.00	0.00	0.00	0.00	00	0.09
	E	0.00	0.00	0.00	0.09[c]	0.00	0.00	0.00	
	F	0.00	0.00	0.00	0.03	0.00			
Hind III	*n*	30	41	49	34	26	41	50	43
	A	0.97	0.98	0.96	0.82	0.88	1.00	0.98	0.98
	B	0.00	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00
	C	0.03	0.00	0.02	0.18	0.04	0.00	0.00	0.00
	D	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	E	0.00	0.00	0.00	0.00	0.00	0.00	0.02[c]	0.00
	F	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.02

Table 1. Cont.

Restriction patterns		POPULATIONS							
		U.S.A.					Mexico		
		COR	ANK	WES	TIF[1]	MOT[1]	ZAR	HER	DEL
Hinf I	*n*	33	48	48	34	26	44	48	42
	A	1.00	0.98	0.96	0.94	1.0	0.96	0.98	0.93
	B	0.00	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00
	C	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.05
	D	0.00	0.00	0.00	0.00	0.00	0.00	0.02[c]	0.00
	E	0.00	0.00	0.00	0.00	0.00	0.02[c]	0.00	0.00
	F	0.00	0.00	0.00	0.00	0.00	0.02[c]	0.00	0.00
	G	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.02
	H	0.00	0.00	0.00	0.03[c]	0.00			
Hpa I	*n*	28	53	43	34	50	34	30	37
	A	0.89	0.87	0.89	0.88	0.94	0.85	0.90	0.94
	B	0.11	0.13	0.09	0.12	0.08	0.15	0.10	0.06
	A/B	0.00	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00
Hpa II/	*n*	28	48	48	33	26	47	55	50
Msp I	A	0.60	0.58	0.77	0.54	0.65	0.77	0.73	0.78
	B	0.36	0.42	0.23	0.46	0.35	.21	0.25	0.18
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02
	D	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02
	E	0.04[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mbo I/	*n*	32	44	49	34	18	48	52	45
Sau3A I	A	0.85	0.88	0.96	0.76	0.94	0.86	0.90	0.89
	B	0.00	0.02	0.00	0.06	0.00	0.02	0.02	0.00
	C	0.03	0.02	0.00	0.03	0.00	0.00	0.00	0.00
	D	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	E	0.03	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	F	0.06	0.00	0.04	0.06	0.00	0.10	0.04	0.09
	G	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	H	0.03	0.02	0.00	0.00	0.00	0.00	0.02	0.00
	I	0.00	0.00	0.00	0.00	0.00	0.02[c]	0.00	0.00
	J	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	K	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02[c]
	L	0.00	0.00	0.00	0.03	0.06	0.00	0.00	0.00

Table 1. Cont.

Restriction patterns		POPULATIONS							
		U.S.A.					Mexico		
		COR	ANK	WES	TIF[1]	MOT[1]	ZAR	HER	DEL
Mbo II	*n*	26	54	39	33	26	31	35	45
	A	0.96	0.94	0.92	1.00	0.92	0.94	0.97	0.92
	B	0.00	0.00	0.03[c]	0.00	0.00	0.00	0.00	0.00
	C	0.04	0.06	0.05	0.00	0.08	0.06	0.03	0.04
	D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04[c]
Rsa I	*n*	18	42	44	34	26	40	32	44
	A	0.94	0.87	1.00	0.97	1.0	0.97	0.94	0.98
	B	0.06	0.00	0.00	0.00	0.00	0.03	0.00	0.00
	C	0.00	0.02	0.00	0.00	0.00	0.00	0.06	0.02
	D	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	E	0.00	0.05	0.00	0.03	0.00	0.00	0.00	0.00
	F	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
	G	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
Sau96 I	*n*	26	54	38	34	26	38	36	51
	A	0.92	0.94	0.95	1.0	1.00	1.00	1.00	1.00
	B	0.04	0.02	0.05	0.00	0.00	0.00	0.00	0.00
	C	0.04[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	0.00	0.04[c]	0.00	0.00	0.00	0.00	0.00	0.00
Sca I	*n*	32	44	50	34	26	38	44	46
	A	1.00	0.96	1.00	1.00	1.00	0.97	0.98	1.00
	B	0.00	0.00	0.00	0.00	0.00	0.00	0.02[c]	0.00
	C	0.00	0.02	0.00	0.00	0.00	0.03	0.00	0.00
	D	0.00	0.02[c]	0.00	0.00	0.00	0.00	0.00	0.00
Ban II	*n*	15	16	16	-	-	18	17	17
	A	1.00	1.00	1.00			1.00	1.00	1.00
EcoR V	*n*	14	16	16	-	-	11	11	10
	A	1.00	1.00	1.00			1.00	1.00	1.00
Fnu4H I	*n*	8	8	8	-	-	10	9	10
	A	1.00	1.00	1.00			1.00	1.00	1.00
Hae III	*n*	13	13	9	-	-	18	8	9
	A	1.00	1.00	1.00			1.00	1.00	1.00
Pvu II	*n*	25	22	26	-	-	5	4	5
	A	1.00	1.00	1.00			1.00	1.00	1.00

Table 1. Cont.

Restriction patterns		POPULATIONS							
		U.S.A.					Mexico		
		COR	ANK	WES	TIF[1]	MOT[1]	ZAR	HER	DEL
Scrf I	*n*	15	17	16	-	-	18	14	18
	A	1.00	1.00	1.00			1.00	1.00	1.00
Sst I	*n*	16	19	14	-	-	18	18	18
	A	1.00	1.00	1.00			1.00	1.00	1.00
Xba I	*n*	12	16	13	-	-	12	10	13
	A	1.00	1.00	1.00			1.00	1.00	1.00

[a]COR = Corvalis, Oregon; ANK = Ankeny, Iowa; WES = Weslaco, Texas; TIF = Tifton, Georgia; Mot = Mountain City, Georgia; ZAR = Zaragosa, Coahuila, Mexico; HER = Hermosillo, Sonora, Mexico; DEL = Delicias, Chihuahua, Mexico.

[b]Overwintering populations collected as pupae.

[c]Indicates private restriction patterns (observed only in single populations).

Table 2. Guide to assigning H. zea individuals to one of the seven major haplotype categories based on restriction patterns of six restriction enzymes.

Hap. Cat.	Hpa I Msp I	Hpa II Sau3A	Mbo I	Mbo II	Rsa I	Sau96 I	Others*
1	O	V	V	V	V	V	
2	A	O	V	V	V	V	
3	A	A	O	V	V	V	
4	A	A	A	O	V	V	
5	A	A	A	A	O	V	
6	A	A	A	A	A	O	
7	A	A	A	A	A	A	

O (other) = any pattern other than A
V (variable) = any pattern including A
* Once mtDNA RFLP patterns of a moth are assigned to one of the above 7 categories, patterns with Ase I, Dra I, EcoR I, Hind III, Hinf I, and Sca I are used to assign a 2-digit haplotype number. When necessary, Alu I pattern can be used to assign a 3-digit haplotype to each moth.

Matching Haplotypes of Immigrant Moths to Those from Suspected Geographic Source: Table 5 illustrates, how the statistical and probability theory when applied to mtDNA haplotypes alone or to a combination of haplotypes and private allozymes can be used to identify the geographic source of migrant moths. We have used data from only two populations for this purpose. Analysis of data on the remaining 6 populations is in progress.

Let us suppose that moths have appeared in Tifton GA in early spring at a time when the progeny from the overwintering resident population has not yet appeared or appeared in low numbers. Let us further presume that the suspected source of migrants is the eastern seaboard of Mexico (represented by Zaragosa; Fig. 1). In such a situation, there should be a 53% probability (based on preliminary haplotype data in Table 4) that we would observe one or the other of the 14 Zaragosa-specific unique haplotypes. In a sample of 50, about 27 moths are expected to have one or the other of the fourteen Zaragosa-specific haplotypes. Use of private allozymes (manuscript in preparation) can improve the effectiveness of mtDNA markers to identify source of migrants. If both mtDNA RFLP and allozyme markers were used (presuming freq. of private allozyme as 0.15), the probability of identifying the source of migrants would increase from 0.53 to 0.68. Table 5 also shows the probability calculations for samples containing different proportions of resident and immigrant moths.

Variability at Nuclear Repetitive Sequences: Preliminary experiments were conducted to estimate the number of restriction sites in rDNA repeat of H. zea for some commonly used restriction enzymes and to select appropriate enzymes for analysis of rDNA variation in wild populations. The restriction digests of total cellular DNA were probed with a clone of the mosquito (*A. quadrimaculatus*) rDNA repeat unit. Of the twenty-two enzymes tested, five (BamH I, EcoR I, Hpa I, Sst I, and Xba I) produced one major band each; Pst I - 2 bands; Sma I - 3 bands (2 major and 1 minor); four (Bgl II, Cla I, Dra I, and Sal I) produced 4 bands each. Of these four enzymes, Bgl II and Cla I produced 3 major and 1 minor band each; Dra I produced 2 major and 2 minor bands; and Sal I produced 2 major and 2-3 minor bands. Six enzymes (Ava I, Hae III, EcoR V, Rsa I, Sau3A I and Sau96 I) produced 6 or more bands. In addition, these preliminary studies showed that 5 enzymes (Alu I, Apa I, Hind III, Pvu II and Xho I and no restriction site (each produced one band and the same size as observed in uncut total DNA. Upon reprobing the filters (previously used for mtDNA RFLP analysis) for rDNA variability studies it was found that Sau3A yielded 6 fragments, but no intra- or interpopulation differences were observed. Further work on filters containing digests with the remaining 12 enzymes is in progress. Preliminary data on a limited sample using M13, and four anonymous repetitive clones revealed ladder like patterns. These studies are being extended to 5 populations (including 2 resident populations) from the U.S. and 3 from Mexico.

Table 3. Guide to identification of mtDNA haplotypes of *H. zea*.

Haplotype	HpaI MspI	HpaII/ Sau3A	MboI/	MboII	RsaI	Sau96I	AseI	DraI	EcoRI	HindIII	HinfI	ScaI
1.1	B	A	A	A	A	A	A	A	A	A	A	A
1.2	B	B	A	A	A	A	A	A	A	A	A	A
1.3	B	A	C	A	A	A	A	A	A	A	A	A
1.4	B	A	D	A	A	A	A	A	A	A	A	A
1.5	B	A	F	A	A	A	A	A	A	A	A	A
1.6	B	A	H	A	A	A	A	A	E	A	A	A
1.7	B	A	J	A	A	A	A	A	A	A	A	A
1.8	B	A	A	A	F	D	A	A	A	A	A	D
1.9	B	A	A	A	G	D	A	A	A	A	A	C
1.10	B	A	A	A	A	A	A	A	E	A	A	A
1.11	C	A	A	A	A	A	A	A	A	A	A	A
2.1	A	B	A	A	A	A	A	A	A	A	A	A
2.2	A	B	B	A	A	A	A	A	A	A	A	A
2.3	A	B	H	A	A	A	A	A	A	A	A	A
2.4	A	B	A	A	H	A	A	A	A	A	A	A
2.5	A	B/A	A	A	A	A	A	A	A	A	A	A
2.6	A	B	A	A	A	A	A	A	E	A	A	A
2.7	A	B	A	A	A	A	A	A	A	C	A	A
2.8	A	B	A	A	A	A	A	A	A	A	B	A
2.9	A	C	A	A	A	A	A	A	A	A	A	A
2.10	A	D	A	A	A	A	A	A	A	A	A	A
3.1	A	A	B	A	A	A	A	A	A	A	A	A
3.2	A	A	C	A	A	A	A	A	A	A	A	A
3.3	A	A	E	A	A	A	A	A	C	E	G	B
3.4	A	A	F	A	A	A	A	A	A	A	A	A
3.5	A	A	H	A	A	A	A	A	A	A	A	A
3.6	A	A	H	A	A	A	A	B	A	A	A	A
3.7	A	A	L	A	A	A	A	B	F	A	H	A
3.8	A	A	B	A	E	A	A	A	A	A	C	A
4.1	A	A	A	B	A	A	A	A	A	A	A	A
4.2	A	A	A	C	A	A	A	A	A	A	A	A
4.3	A	A	A	C	A	B	A	A	A	A	A	A
4.4	A	A	A	C	A	A	A	A	A	A	E	A
4.5	A	A	A	D	A	A	A	A	A	A	A	A

Table 3. Cont.

Haplotype	HpaI MspI	HpaII/ Sau3A	MboI/	MboII	RsaI	Sau96I	AseI	DraI	EcoRI	HindIII	HinfI	ScaI
5.1	A	A	A	A	B	A	A	A	A	A	A	A
5.2	A	A	A	A	B	A	A	A	A	A	A	C
5.3	A	A	A	A	C	A	A	A	A	A	A	A
6.1	A	A	A	A	A	B	A	A	A	A	A	A
6.2	A	A	A	A	A	C	A	A	A	A	A	A
7.1	A	A	A	A	A	A	B	A	A	A	A	A
7.2	A	A	A	A	A	A	A	B	A	A	A	A
7.3	A	A	A	A	A	A	A	A	B	C	A	A
7.4	A	A	A	A	A	A	A	A	B	A	C	A
7.5	A	A	A	A	A	A	A	A	A	C	A	A
7.6	A	A	A	A	A	A	A	A	A	A	C	A
7.7	A	A	A	A	A	A	A	A	A	A	F	A
7.8	A	A	A	A	A	A	A	A	A	A	A	A

Discussion

mtDNA Haplotypes as Genetic Markers: Our results show that differences among distant populations of *H. zea* are limited to low-frequency haplotypes. This is not unexpected, because in many animal taxa, mitochondria have been shown to undergo a rapid rate of sequence and structural evolution. For example, 133 different mtDNA types were obtained by analyzing mtDNA of 147 humans in five European populations using 12 restriction enzymes (Cann et al. 1987). Analysis of populations from Asia, West Africa, America, and Middle East showed similar results (from Excoffier et al. 1992).

Table 4. Frequency distribution of mtDNA haplotypes in resident population (from overwintering pupae) of *H. zea* from Tifton, GA. Data from Zaragosa, Mexico (from pheromone traps) are included for comparison.

Haplotype	U.S.A. TIF	MEXICO ZAR	Haplotype	U.S.A. TIF	MEXICO ZAR
n	34	35	*n*	34	35
1.3.2	0.03	0.00	3.8.9	0.03	0.00
1.4.1	0.00	0.06	4.2.2	0.00	0.03
1.4.2	0.00	0.08	4.4.1	0.00	0.03
1.5.2	0.03	0.00	5.2.1	0.00	0.03
1.6.2	0.03	0.00	7.3.1	0.03	0.00
1.10.2	0.03	0.00	7.3.2	0.03	0.00
2.1.1	0.00	0.06	7.5.1	0.03	0.00
2.1.2	0.32	0.17	7.5.2	0.03	0.00
2.1.5	0.00	0.06	7.7.1	0.00	0.03
2.2.2	0.03	0.00	7.8.-	0.00	0.17*
2.5.2	0.03	0.00	7.8.1	0.23	0.14
2.6.2	0.03	0.00	7.8.2	0.00	0.03
2.7.2	0.03	0.00	7.8.5	0.00	0.03
2.10.-	0.00	0.03	7.8.6	0.00	0.06
3.5.2	0.03	0.00			
3.7.2	0.03	0.00			

* Alu I pattern not analyzed, therefore 7.8.- could contain 7.8.1 or 7.8.2 or both. The third digit of Alu I: 1=A; 2=B; 3=C; 4=D; 5=E; 6=F; 7=G; 8=H; 9=I.

Table 5. Illustration of potential effectiveness of mtDNA haplotype markers for identification of immigrant *H. zea* moths. R=Resident Population from Tifton, GA, I=Immigrant Population, probability values used (P^1; mtDNA haplotypes, P^2: mtDNA + allozyme[a]).

	Probability of Diagnosis (P)		
R	I	P^1	P^2
0	1	0.53	0.68
1	1	0.27	0.34
2	1	0.18	0.23
3	1	0.13	0.21

[a]Unpublished data.

Therefore, for mtDNA haplotypes to be effective markers, powerful statistical and probability methods must be used to identify the geographic and host plant origin of immigrant *H. zea* populations.

It is widely recognized among population geneticists that the level of genetic differentiation in highly mobile insect species is far lower than that in less mobile species. This will be particularly true for the nuclear genome, which undergoes repeated recombination events. To the contrary, uniparentally inherited mitochondria reveal greater heterogeneity in a species than the nuclear genes. Our results substantiate this expectation.

The mitochondrial genome consists of a single circular DNA molecule, which is inherited maternally. Unlike the nuclear genome, which is affected by sexual recombination and gene flow between populations, mitochondria are free from such genetic homogenization effects (Brown, 1985; Wilson et al. 1985). Consequently, mtDNA show less intrapopulational and more interpopulational variation than autosomal nuclear loci. Therefore, mtDNA RFLP data can be used for tracing maternal lineages during analysis of changes in genetic make-up of resident noctuid populations during the migration period.

Use of mtDNA haplotype data for the identification of the geographic source of migrant insects often relies on the probability of specimen match based on the additive property of allelic frequencies of independent genetic loci. Such an approach was used to identify discrete subpopulations in *Anopheles* mosquitoes (Narang et al. 1990). In our ongoing study of *H. zea*, the frequency of the unique mtDNA haplotypes in resident larval populations infesting crops and suspected immigrant adult moths will be used for the probability statistics as illustrated in Tables 4 and 6.

Population Genetics Literature on H. Zea and Other Moth Spp: Except for our preliminary work on mtDNA polymorphism in *H .zea*, very little is known of the type, nature, and extent of genetic variability in natural populations of this species (and *H. virescens*). Preliminary isozyme studies (only 10 loci) on these two species by Sluss et al. (1978) and Sluss and Graham (1979) showed high levels of genetic variability in both species. However, allelic differences among populations were not significant, indicating high migration rate. Contrary to the above observations, Sell et al. (1974 a,b, 1975) reported significant allele frequency differences among four populations of *H. zea* collected from different hosts (corn vs a leguminous plant). Therefore, the Est-II can be used to trace host-specific migration, but there is no report on the use of this locus in migration studies by these authors to date. More recently, in a limited survey of a few *H. zea* populations, Mallet et al. (1993) failed to observe the level of electrophoretic variability reported either by Sluss et al. (1978) and Sluss and Graham (1979) or by Sell et al. (1974 a, b, 1975).

In studies on two Australian species, *Helicoverpa armigera* and *Heliothis punctigera*, Daly and Gregg (1985) reported significant heterogeneity in gene (electrophoretic) frequencies in natural populations of each species, but found an overall low level of genetic differentiation. They concluded that the effective population size of these species in Australia is large, and that significant gene flow probably occurs between widely separated regions.

Emerging DNA Fingerprinting Technologies: The emerging genetic technologies provide an opportunity to determine population variability and the interaction of populations on a regional scale due to imgration. Until 1988, DNA polymorphisms could be only be determined by the RFLP method, however, introduction of the polymerase chain reaction (PCR) using thermostable DNA polymerase enables the analysis of highly variable, simple-sequence, tandem repeats (microsatellite sequences) (Weber, 1990). Microsatellite DNA, regardless of species and repeat length, exhibits variation in the number of units per block of repeats (Weber and May, 1989 and Smeets et al. 1989).

In addition probes from multigene families can prove useful for individual and population marker analysis. An example of such a family are genes coding for some esterases. For instance, *Culex* spp. and aphids (*Myzus persicae* and a tobacco-feeding species, *M. nicotianae*), exhibit closely related families of esterases, which

confer insecticide resistance (in *Culex* spp. by detoxification), (review by Devonshire et al. 1992). In resistant insects, there is an increased synthesis of enzyme by amplification of the esterase gene (up to 500-fold increase in gene copy number in *Culex* spp.) (Mouches et al. 1987). These amplified esterase genes are inherited as an allele and are likely to be in a tandem array with little to no recombination among them (Ferrari and Georghiou, 1991). Sequence data indicate considerable homology of the *Culex* esterases with esterases of *Heliothis* (Pasteur, 1990). Therefore esterase DNA sequences can be useful probes for DNA typing of moths. Another example of a multigene family is the α-amylase locus in *Drosophila* spp. It consists of 3 genes located adjacent to each other (one highly expressed gene, one lowly expressed gene and a pseudogene). The three genes are extremely similar in DNA sequence. Other possible probes could include genes for hormones. For example, genes coding for the neuropeptide prothoracicotropic hormone (PTTH) have been extensively studied in *Bombyx mori* and *Manduca* spp. The bombyxin gene is a member of a multigene family consisting of at least 5 genes (Kawakami et al. 1989, Iwami et al. 1989). Variations in these and other multigene families could be useful.

Summary

There is considerable circumstantial evidence supporting south to north long-range spring migration of noctuid pests, however, methods to determine the origin of migrants are lacking. In an attempt to solve this problem, we conducted population DNA fingerprinting studies on *H. zea* collected from several locations. Three wild populations from Mexico (Zaragosa, Delicias and Hermosillo), and five from the U.S. (Corvalis, OR, Ankeny, IA, and Weslaco, TX and two resident populations from Tifton and Mountain City, GA) were analyzed for mitochondrial DNA (mtDNA) restriction differences and variation for 6 other repeated sequences. The goal was to determine whether some of the differences could be used to distinguish immigrant from resident populations.

Of the 74 mtDNA RFLP patterns observed, 43 (58%) were characteristic of the two regions (present in either Mexico or the U.S.). Some of the remaining 31 (42%) patterns showed frequency differences among populations.

We illustrate how statistical and probability theory, when applied to low-frequency private mtDNA haplotypes alone or to a combination of haplotypes and other markers (private allozymes and microsatellite DNA fingerprints), may be used to identify geographic sources of migrant moths. A comparison of mtDNA haplotypes of resident moths from Georgia with those adults from traps from a site in northeastern Mexico shows that if migrant moth appear in Tifton, GA from Zaragosa early in the spring there is a 53% probability that migrants will be identified by revealing at least one of the 14 Mexico-specific mtDNA haplotypes.

The ongoing analysis of allozyme and microsatellite polymorphism will further enhance the effectiveness of this probability method for genetic identification of migrants and determination of their contribution to local population dynamics.

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APPENDIX E. REVIEW TEAM REPORT AND RECOMMENDATIONS

Summary

The review team recommends that ARS be involved in creating, testing, and modifying site specific management schemes, but that it do so with the overall goal of developing area wide programs. In our view, there may well be a need for different area wide management schemes, depending on the environment, cropping systems and management opportunities. A careful analysis needs to be made of the usefulness of multiple approaches to area wide *Heliothis/Helicoverpa* (*H/H*) management.

We recommend that you actively formulate an information transfer process. This would likely mean the formation of partnerships and alliances with other agencies such as AES and CES. Stakeholders need to be assembled and brought into the planning process in the relatively near future. This is needed in order to create a broad support base for and general understanding of the program.

Biological discoveries should dictate the evolutionary directions and pace of the area wide management program. Such discoveries should be viewed from the perspective of how they fit within the ecology of the system. We conclude that it is time to sharpen the focus while broadening the vision. We recommend that the program undertake a critical assessment of sampling procedures and needs, especially in the context of an area wide approach.

ARS should examine its comparative advantage and, on that basis, deploy resources. It must do so, however, within the context of other active players so that the overall objectives are not lost. We strongly recommend that additional resources be directed at elucidating the underlying causal mechanisms of *H/H* migration.

The very positive view put forth by the group on the use of biological control as a population suppressant, especially in source population reduction approaches, is admirable. We share that view.

When developing an area wide pest management program, the total knowledge and personnel base must be brought to bear on formulating and executing the highest quality program.

This program is important but demands tighter coordination to have potential as an area management program. The review team strongly recommends that an *H/H* program manager be appointed to oversee this important activity at the earliest possible time. This leader should devote his/her energies entirely to the conceptual and operational aspects of the program.

The Review

The members of the review team individually and collectively express their thanks for the opportunity to participate in the conference. As a review team, we found it most educational and stimulating to learn about the developments in the program. The secluded setting created an opportunity for meaningful dialogue and interaction. the review team recognizes the tremendous effort and coordination that went into planning and developing the program and the local arrangements logistics that made the running so smooth and cordial. We compliment your for a job well done.

The team was impressed with the breadth of the research effort and the foresight in developing the basis for the program which can potentially lead to a unique approach to an area wide *H/H* management program. We comment the leadership ad the individual scientists for their often innovative efforts in this direction. Further, we were extremely pleased to note that a fundamental research program is supporting the applied initiative.

With a few exceptions as noted in the discussion below, the Action Areas are individually making excellent progress on the Arrays. The establishment of Action Areas and arrays for elucidating them is a strong indication of the

strength of the initiative. Further, there is good leadership collectively and within each Action Area. What is unclear is the status of the cross-cutting issues, the integration of discovery and development across Action Areas. Each Action Area would appear to be maximizing its development in a given area instead of optimizing its development or examining how it relates to other Action Areas and to an overall management strategy. At this point, determining what it takes to move the total program forward is much more important than allowing the program to focus on the objectives of individual scientists or on single Action Area programs. We recognize that engaging "a managed research thrust" is a delicate issue, one fraught with difficulties as it relates to scientific inquiry and creativity. However, some form of "a managed research thrust" would, we believe, facilitate the development of the information base required to implement an area wide *H/H* management program.

Presently, there appears to be a mixed program activity. The various parts of the total program have set their own agendas and are internally well coordinated, but a targeted, directed approach from program perspective seemed to be lacking. If the goal is developing an area wide *H/H* program, then a specifically targeted and directed approach seems warranted. An industry approach, heavily goal oriented, would mandate that each experiment be justified as contributing to the overall goal. To institute a program of that type, a benevolent czar with a small management advisory group should be considered. They would be responsible for a final product and a team directed to developing agreed upon objectives should be considered as an operational mode.

While we understand the importance of a targeted research approach, we also caution that one cannot under value the potential for contribution that unique, breakthrough, pioneering research might make to the program. Some provision or accommodation must be made in the research mode to encourage some entrepreneurial, breakthrough opportunities.

As you develop an area wide *H/H* program management plan, we recommend consideration of an industry-type model. Industry would generally identify someone as a "product manager." You have a product, that is, area wide *H/H* management. We suggest that you examine "parallel" instead of "series" development processes. In this approach, there is an optimization of the components to the whole rather than maximization of components for individual operation. It requires individual adjustment and sacrifice. Within the context of the *H/H* program, for example, the host resistance effort might be placed in a more complimentary mode with the efforts in the biological control effort, and vice versa. Larval growth and development retardation by host resistance might enhance biological control because larvae are exposed to natural enemies for a greater period of time. The question, then, is how to capitalize on the strengths of this interaction. The review team suggests that an *H/H* program manager be appointed to oversee this important activity. This leader should devote his/her energies entirely to the conceptual and operational aspects of the program.

We strongly embrace the concept of area wide pest management but believe its limits, temporally and spatially, need to be clearly defined. What does "area" mean? In our view, an area wide approach would involve a continuum of activity from discovery to user friendly delivery. ARS has many strengths. It must identify its comparative advantage in that continuum, define its limits, and identify the parts of this continuum where is should contribute.

The applicability of the research generated by the *H/H* action plan to a field by field pest management scheme needs to be assessed. How might such a plan impact the current practice and future potential of site specific pest management? It is extremely important to evolve a control strategy for the future; it is also critical for the producer to have the best information and technology during the time that "better approach" is being developed. The producer can and should help identify which tactics are to be used in the interim, until more comprehensive programs are deployed. For the *H/H* management program, have your customers been identified? Are you wholesalers or retailers? How do you perceive your interaction with other state scientists and state and county extension staff? We perceive an important need for cross fertilization and integration of activities. We recommend that you examine research objectives and developments in the context of these questions and actively formulate an information transfer process. This could mean the formation of strategic partnerships with other agencies.

The Action Plan presented at Junction needs two additional chapters, each with multiple scientific and agency authors. One of the two chapters relates to linkage/integration issues and the other to implementation systems issues. There is need for a strategic management, development, and deployment plan for any area wide pest management activity.

The review team recommends that you create, test, and modify site specific management schemes, but that you do so with the overall goal of developing area wide programs. In our view, there may well be a need for different area management schemes, depending on the environment, cropping systems and management opportunities. In the proposed scenario, multiple tactics in various combinations in different areas must be evaluated to determine the most effective, cumulative, or synergistic tactics to employ in an area wide program. It seems likely that multiple options will be required for area wide pest management programs. These options are likely to be locale specific; for example, a plan for south central Mississippi may differ from one for the Carolinas. Programs will differ enormously if they are implemented in a pre- or post-boll weevil suppression area. A careful analysis needs to be made of the usefulness of multiple approaches to area wide *H/H* management.

Prioritizing the research agenda is critical when focusing on area wide pest management. Presently, individual project justification is apparent in the *H/H* program. If the objective is to move towards area wide management in the future, then a strategic plan for research needs to be generated; this plan must be bought into by multiple participants and agencies. One need to move away from applying existing, in place science and technology towards seeking appropriate science and technology that is needed to expedite the program.

The development of an area wide program must involve the consideration of the total knowledge base for *H/H*. The Junction meeting dealt only with ARS generated research, and that may well have been the intended purpose of the meeting. It must be pointed out, however, that additional valuable knowledge exists within other agencies, practitioners, industries, and university research efforts. When developing an area wide pest management program, the total knowledge and personnel base must be brought to bear on formulating and executing the highest quality program.

Given the above statement, stakeholders need to be assembled and brought into the planning process in the relatively near future. This will create understanding and a support base for the program. In our view, the success of an area wide *H/H* management program is dependent upon generating significant stakeholders buy-in and enthusiasm. They must feel that they are part of the activity. The stakeholders must get together and jointly plan the activity. It needs to be a partnership meeting that involves federal, state, local area personnel, consultants, practitioners, and producers in a joint effort to discuss the goals, objectives, operational approaches, and implementation aspects of a potential plan.

The biology must do the leading in any research and management effort. To borrow a phrase that was used in a presentation, "go with the flow," conveys much about the process. The emphasis in the case of an area wide *H/H* management program should be on the "biology." Biological discoveries should dictate the evolutionary directions and pace of the program. Such discoveries should be viewed from the perspective of how they fit within the ecology of the system. Presently, we have the impression that the program is throwing traditional sub-discipline technologies at the problem and hoping for a settling out of relevant facts. Consider the question, "What are the critical, scientific and technical needs, based on an understanding of biological vulnerabilities of *H/H*, that will facilitate an area wide pest management program?" To paraphrase another statement, it appears to be time to "sharpen the focus while broadening the vision."

Specific Observations and Recommendations

Have you considered how the effective removal or neutralization of *H/H* as a pest may impact the ecology of the system?

What is the relevant or appropriate role for ARS in the *H/H* program as it relates to the evaluation of commercial products, such as pesticides and Bt engineered plants? In considering that point, it would seem appropriate to address the question, "Is this, or any other, commercial product technology projected to be an integral part of a designed area wide management plan?" The critical point is that ARS should examine its comparative advantage and, on that basis, deploy resources. It must do so, however, within the context of other active players so that the overall objectives are not lost.

There appears to be considerable difference of opinion on the adequacy and the appropriateness of population assessment technology. Basic to quantifying pest dynamics is a reliable sampling method, whether the need is for site specific, local, or area wide quantification. Some studies focus on *H/H* movement and migration; these must consider that aspect of biology in the context of management. We recommend that the program undertake a critical assessment of sampling procedures and needs, especially in the context of an area wide approach. Current sampling is focused on adult males even though it is the larvae that cause the damage and the females that lay the eggs. Should a significant effort be focused on developing sampling methodology for females and larvae?

Biological control appears to be rather promising for *Helicoverpa*, particularly the nematode aspects. However, we caution that the basic biological understanding of the nematode is not yet great enough to incorporate it into an area wide management program. Also of significance is the use of genetics to craft parasitoids that are more competitive in specific instances where pests are to be controlled outside of traditional fields. The very positive view put forth by the group on the use of biological control as a populations suppressant, especially in source population reduction approaches, is admirable. The development of genetic markers is seen as an important component of the project. It is viewed as having the potential to be an important tool for understanding how genes transfer among populations as well as the obvious use in identifying the origin or source of populations.

While the review team sees promise in the *Bacillus thuringiensis* (*Bt*) transgenic plants, it also cautions that *Bt* transgenic plants may well be the insecticide treadmill of the future. Given the widespread transgenic manipulation involving *Bt*, there is serious question as to whether we have the ability to critically deploy this hand-crafted biological control agent. Without critical analytical deployment, it is quite likely to go the route of traditional insecticides.

There were several mentions of the uniqueness of this species complex, namely, a very wide host range, considerable genetic plasticity, and unusual mobility. One unique characteristic not mentioned was that while these insects are generalists, they are also specialists when it comes to the stage of the plant on which they prefer to feed, that is, fruiting structures. Can this aspect of the pests' biology be used to help develop an area wide management program?

The project on the migration of *H/H* is at the cutting edge of science and technology. In our view, it represents one of the most important aspects of the complex biology from the viewpoint of developing area wide pest management strategies. The research has begun to answer questions about the migration event, i.e., when it occurs and how it progresses. We strongly recommend that additional resources be devoted to this project to elucidate the underlying causal mechanisms of *H/H* migration.

The team views a need for an enhancement of the effort in application technology that is directed to the new base of discovery on tactics and their utilization in a management program.

As we engage new area wide management programmatic thrusts it is important to expand our horizons, cultivate and enhance lines of communication and cooperation between state and federal agencies, producers, and advisors in order to deal effectively in the arena.

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